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CONTAGIOUS BOVINE PLEURO-PNEUMONIA. NOTE ON EXPERIMENTAL REPRODUCTION AND INFECTION BY CONTACT.

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ALTHOUGH it is now 36 years since Nocard and Roux were able by the aid of an ingenious technique to demonstrate the presence of an organism in the lymph from cases of contagious bovine pleuro-pneumonia, it has not yet been possible to satisfy Koch's postulates, *i.e.*, to demonstrate conclusively the ætiological significance of the organism. All attempts to reproduce the disease by inoculation of virulent thoracic lymph or pure cultures of the organism have failed, whatever the method of inoculation employed—whether subcutaneous, intravenous, intratracheal, intraperitoneal, intrapleural, or intracerebral. Indeed one has at times been driven to speculate whether the explanation of these repeated failures might not be that the disease was after all a true virus disease, with the familiar organism playing the rôle of a mere secondary invader or *bacille de sortie*.

A striking feature in the relationship between the organism and the disease is the fact that the organism when injected subcutaneously in culture or lymph, even in minute amount, will provoke intense œdematous reactions which frequently terminate fatally. It has been claimed with some justification that the essential nature of the inflammatory process in the subcutaneous tissues is identical with that of the lung changes in a natural case, but again it is astonishing that even in fatal cases and even where the infective material has been inoculated into the pleural cavity the lungs have never been attacked. There is, however, a certain parallelism in the relations that obtain between the pneumonic form of bovine hæmorrhagic septicæmia and its causal organism *Pasteurella boviseptica*, although in this instance the lung changes simulating those of pleuro-pneumonia are only rarely found in natural cases of the disease, and subcutaneous swellings of the type provoked by inoculation of the organism are the rule in natural infections.

Little is known concerning the spread of bovine pleuro-pneumonia under natural conditions and Dahmen and Ziegler

(1929) adequately sum up the position in the sentence " Der natürliche Infektionsweg ist noch nicht genau bekannt." The incubation period is generally stated to be from two to eight weeks, but apart from the single successful experiment of Chauveau and Nocard, in which the infection was obtained by joining together the heads of an infected and a healthy animal with a tube of linen, there is no record of contact infection ever having been induced under controlled experimental conditions.

In spite of these glaring deficiencies in our knowledge of the ætiology of the disease it has been possible to stamp out bovine pleuro-pneumonia in most parts of Northern Europe by the slaughter of infected and in-contact animals. Where large expanses of infected territory in such countries of Africa are to be administered, and particularly in those areas inhabited by nomadic pastoral tribes, the adoption of the slaughter policy is unfortunately not practicable; and it becomes necessary to rely upon the operation of quarantine or segregation measures, coupled with vaccination, to control the spread of a disease in which a proportion of clinically recovered animals remain infective for prolonged periods. It is under such conditions that the paucity of accurate experimental data on the disease is most keenly felt; and when one has also the added problem of protecting clean areas (under both white and native settlement) against frequent re-invasion from adjacent infected native territories, the need for further reliable information on the nature of the infection becomes even more pressing.

Of the serological tests that have so far been devised for the detection of carrier-animals or " lungers," none has been an unqualified success under African conditions, and although certain factors to explain their failure are brought to light in this note it is extremely doubtful, for the reasons given, whether it will ever be possible to base control entirely on any diagnostic test.

With the requirements of tropical countries in mind, it was decided to make a further attempt to reproduce the disease artificially and, if possible, to study infection by contact under experimental conditions. The object of this note is to record the progress that has already been made in this direction, and to indicate the technique that has led to successful results.

INTRATRACHEAL INOCULATION.

The first attempts at artificial infection were made by the intratracheal route, using virulent thoracic lymph filtered through a British Berkefeld filter or a Chamberland L7 filter. Tests carried out previously had shown that the organism regularly passes the Berkefeld and is always retained by the L7. The method of inoculation was to puncture the wall of the trachea with a canula or coarse hypodermic needle, and then to pass the needle of the inoculating syringe through the canula and into the

lumen of the trachea. After injection of the inoculum a clean syringe was fitted to the inoculating needle and several c.c. of normal saline were forced through to free it of all traces of virus. It will be observed that in spite of these precautions several of the inoculated animals contracted severe subcutaneous swellings at the site of inoculation.

Two animals were inoculated intratracheally with each filtrate, and three animals inoculated subcutaneously in the shoulder served as controls for the infectivity or sterility of the filtrates. The results of this series of inoculations are tabulated below. In the one positive case, to which a brief reference was made in the Annual Report for 1932, a temperature reaction commenced on the 18th day after inoculation, and in the course of the next few

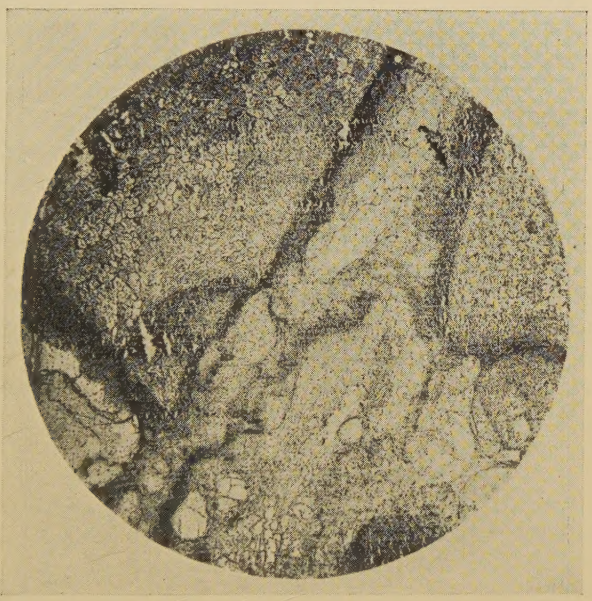


FIG. 1.
Section of lung of X4726. Acute pleuro-pneumonia produced by intratracheal inoculation of filtered lymph. (Hæmatoxylin and Eosin. Low magnification).

days this animal developed an acute pleuro-pneumonia, in which the right lung became almost completely hepatised. There was an extensive pleuritic effusion detectable clinically, and the animal died on the 29th day of experiment.

Post-mortem there was a considerable quantity of exudate in the pleural cavity with fine fibrinous coagula. The affected right lung was enlarged and covered with a thick tough pellicle formed from coagulated and organised pleural exudate.

Macroscopically the lung picture was quite typical of the acute type of disease, with no attempt at resolution, and the characteristic parenchymatous and interstitial changes were most marked. Histologically the lesion was pathognomonic. (Fig. I.)

TABLE I.

Attempted Infection—28.7.32.				*Immunity Test*—29.8.32.			*Agglutinin-Precipitin or Agglutination Tests.*	
No. of Animals.	*Site of Inoculation.*	*Material.*	*Result.*	*Site of Inoculation.*	*Material.*	*Result.*	*1 Month after 1st Inoculation.*	*After Immunity Test Inoculation.*
7332	Intratracheal	Dilute Berkefeld filtrate of lymph from natural case SB3135	No apparent reaction	Subcutaneous shoulder	Berkefeld filtrate of lymph from X4726	No reaction	—	—
4726	,,		Clinical pleuro-pneumonia (cultures +) 27.8.32 †	...		...	...	...
7751	Subcutaneous shoulder		Severe subcutaneous reaction 30.8.32 †	Subcutaneous shoulder		...	...	...
7745	Subcutaneous shoulder		Severe subcutaneous reaction 30.8.32 †	Subcutaneous shoulder		...	...	...
6358	Intratracheal	Dilute L7 filtrate of lymph from natural case SB3135	No apparent reaction	Subcutaneous shoulder	Berkefeld filtrate of lymph from X4726	Mild reaction	—	± to +
7274	,,		No apparent reaction	Subcutaneous shoulder		No reaction	—	++
7331	Subcutaneous shoulder		No apparent reaction	Intratracheal		No apparent reaction	+	+++

† Died. + Positive. ± Doubtfully positive.

A culture of the organism was obtained from a Berkefeld filtrate of the thoracic lymph, and this filtrate also provoked severe reactions that proved fatal in four animals out of six that were inoculated either subcutaneously or into the trachea with escape of some of the inoculum into the subcutaneous tissues. (*Vide* Table II.) Pure cultures were also recovered by direct seeding of the pleural exudate. It will be observed that the L7 filtrate of the same lymph did not give rise to any reactions either in the animal subcutaneously inoculated or in the two inoculated intratracheally. Subsequent immunity tests indicate that some immunity may have been derived from the inoculations of L7 filtrate, since, of six fresh animals inoculated with the Berkefeld filtrate of lymph from 4726 (Table II), four referred to above developed fatal reactions, while of the three animals previously treated with L7 filtrate (Table I) none reacted severely.

The remaining animal in this series, No. X7332, inoculated with a Berkefeld filtrate was also immune, although apparently he did not react to the original intratracheal injection.

I am indebted to Mr. J. R. Hudson for the carrying out of the first series of intratracheal inoculations, which include the only positive transmission by this route.

In the second experiment twelve cattle were inoculated: four intratracheally and two subcutaneously with a Berkefeld filtrate of lymph from X4726; four intratracheally and two subcutaneously with an L7 filtrate of the same lymph. The results of these inoculations are recorded in Table II, together with the results of immunity tests performed on the survivors. No transmissions were obtained in this series, although one animal, X7832, showed considerable congestion and enlargement of the lungs, possibly hypostatic in origin, at death, without any severe subcutaneous reaction at the site of inoculation.

Two further attempts to transmit by intratracheal injection of Berkefeld filtrates of shoulder lymph also failed, although twelve animals were inoculated. In the 2nd, 3rd and 4th series there were four accidents due to infection of the subcutaneous tissue during intratracheal inoculation and in each case the local reaction was responsible for death, but there were no lung changes in spite of the certainty that most of the inoculated virus had been deposited in the trachea.

EMBOLUS PRODUCTION.

Transmission was next tried by intrajugular inoculation of virulent lymph or pure culture included in emboli of 2-3 per cent. agar; the technique follows that devised by Tuttle (1933) for the production and study of pulmonary abscesses in the dog. A measured quantity of lymph or of centrifuged broth culture is mixed with a few cubic centimeters of 10 per cent. agar and is then drawn up into a metal canula of about one-sixteenth of an

	Attempted Infection—29.8.32.			*Immunity Test.*				
							Agglutinin-Precipitin or Agglutination Tests.	
No of Animals.	*Site of Inoculation.*	*Material.*	*Result.*	*Site of Inoculation.*	*Material.*	*Result.*	1 *Month after 1st Inoculation.*	*After Immunity Test Inoculation.*
8068	Intratracheal	Dilute Berkefeld filtrate of lymph from X4726	Severe subcutaneous reaction 22.9.32 †	...	...	...	...	...
8069	"		Severe subcutaneous reaction 25.9.32	...	...	...	...	...
7891	"		No apparent reaction	Subcutaneous shoulder 24.11.32	Pure culture 5th generation X8068	Mild reaction	—	++
7998	"		Severe subcutaneous reaction 17.9.32 †	...	...	...	...	...
8058	Subcutaneous shoulder		Severe reaction 16.9.32 †	...	...	...	+++	...
8051	Subcutaneous shoulder		Mild local reaction	Subcutaneous shoulder 24.11.32	Lymph from X7904	Mild reaction	+++	+++
7743	Intratracheal	Dilute L7 filtrate of lymph from X4726	No apparent reaction	Subcutaneous shoulder 24.11.32	Pure culture 5th generation X8068	Mild reaction	±	+
7761	"		No apparent reaction	Subcutaneous shoulder 24.11.32	Pure culture 5th generation X8068	Marked reaction	—	++
7556	"		" "	Subcutaneous shoulder 24.11.32	Pure culture 5th generation	Marked reaction	±	—
7638	"		" "	Subcutaneous shoulder 24.11.32	Pure culture 5th generation X8068	No reaction	+	±
7832	Subcutaneous shoulder		" "	Subcutaneous shoulder 24.11.32	Lymph from X8068	Very mild reaction	±	...
7790	Subcutaneous shoulder		" "	Subcutaneous shoulder 24.11.32	Lymph from X8068	No reaction*	+	—

+ Positive. ± Doubtfully positive. * Later became negative to serological test 1 month after inoculation.

inch bore that has previously been lubricated with glycerin. The agar is allowed to set. The jugular vein of the animal is punctured with a large bleeding trocar and canula, and after the trocar has been withdrawn the smaller canula containing the embolus is inserted through the large one. The embolus is now forced out into the lumen of the vein by an injection of normal saline from a syringe that has been attached to the small canula by a rubber connection.

Infection with Thoracic Lymph.

Two cattle, Nos. X7927 and X7928, were inoculated in this manner, each with 1 c.c. of thoracic lymph.

X7927 showed a small local swelling during the third week after inoculation but this swelling receded rapidly and had disappeared within five days. During the fourth week after inoculation a temperature reaction developed, but no definite lung changes could be detected by clinical examination. This reaction lasted seven days, the highest morning temperature, 104·2°, being recorded on the 27th day after inoculation (Chart I). On the 16th day, that is just when the subcutaneous reaction was subsiding, the serum of X7927 gave a strongly positive result to the agglutination test. Tested again during the thermal reaction on the fourth week he gave a doubtful result, but again became strongly positive on the sixth week and continued positive until death. Clinically the animal remained apparently healthy for a period of three weeks and was then destroyed for *post-mortem* examination, eight weeks after inoculation and four weeks after the peak of the temperature reaction. At *post-mortem* examination there was no exudate in the thoracic cavity but there was a firm adhesion of the right lung to the chest wall over an area of roughly 2-in. in diameter. Beneath this adhesion lay an encapsuled oval sequestrum measuring roughly $1\frac{1}{2}$-in. × $2\frac{1}{2}$-in. The surrounding tissues for a distance varying from 1-in. to 3-in. from the periphery of the sequestrum showed some slight interstitial thickening, but no definite hepatisation. In the left lung there was one small encapsuled lesion about the size of a pea which contained caseous material. No culture was obtained, but histologically the lesion in the right lung was typical for an organising sequestrum. The site of inoculation was normal.

The companion animal, X7928, never had any local swelling. During the second week after inoculation he developed a febrile reaction which lasted roughly nine days. On the 16th day, *i.e.*, towards the end of the thermal reaction, X7928 gave a strongly positive result to the agglutination test; this result was confirmed several times before he was destroyed. There were no clinical symptoms and the animal remained in fair condition for seven weeks after inoculation and was then destroyed. *Post-mortem* there was no exudate in the thoracic cavity, but on the right side

there were two circumscribed adhesions and lying beneath them were two lesions, each about 3½-in. in diameter, fairly well separated from the healthy tissue. Towards the periphery the tissue of the lesions was organised and straw-coloured, while in the centre it was deep, dull crimson and homogeneous in texture. In the left lung there were three smaller lesions ranging from 2-in. to 3-in. in diameter, and in the largest the tissue had undergone a marked softening. Over each of these lesions there was an adhesion to the parietal pleura. Cultures were not obtained, but histologically the lesions were diagnostic.

Infection with Culture from Thoracic Lymph.

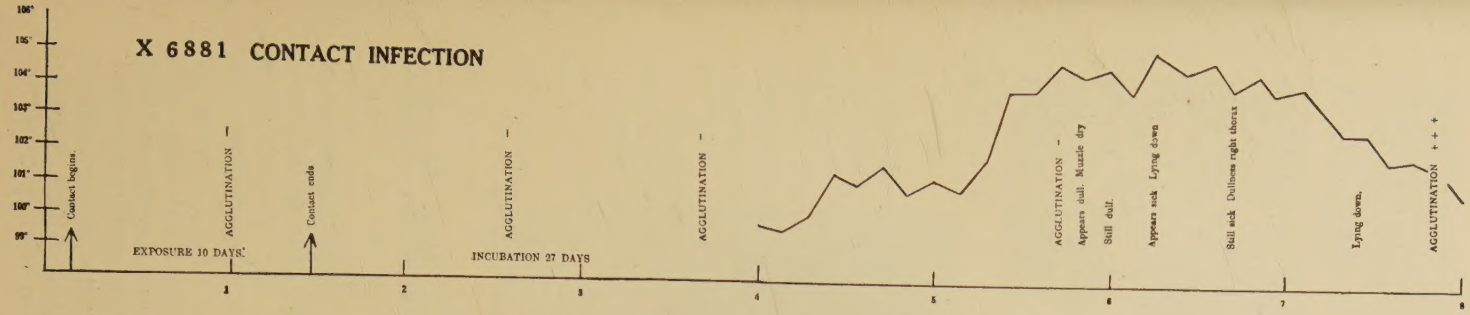
Two animals, Nos. X8082 and X8667, were inoculated in similar fashion with first generation culture from thoracic lymph obtained from a natural case of the disease. X8082 developed a local swelling at the site of inoculation, which persisted from the twelfth to the 21st day. Serum taken from X8082 on the 16th day gave a strongly positive reaction in the agglutination test, and a similar result was obtained with blood drawn on 27th, 40th, 48th and 63rd days. During ten weeks' observation this animal never showed any other reaction to the inoculation, but when destroyed on the 70th day it was found to have one sequestrum in the left lung. Histologically the lesion was diagnostic, but cultures could not be obtained.

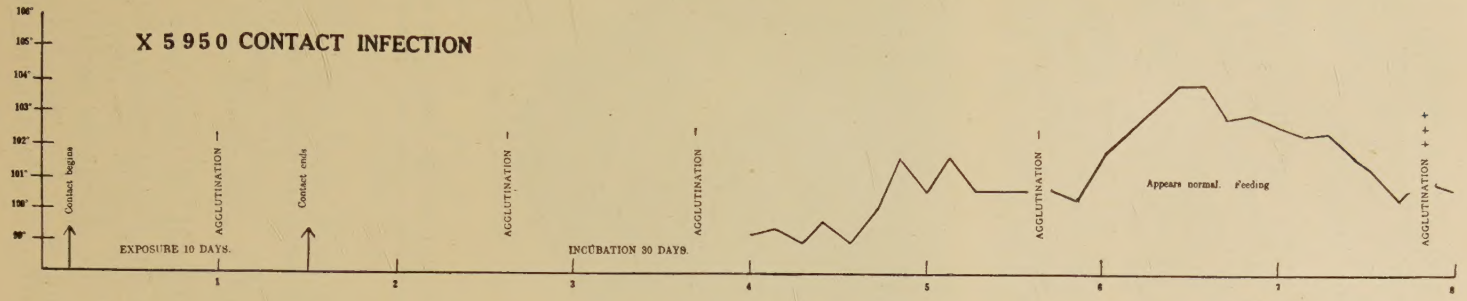
The other animal, X8667, did not react in any way to the inoculation, and gave consistently negative reactions to the agglutination test up to the 70th day, when he was destroyed and found to have completely healthy lungs.

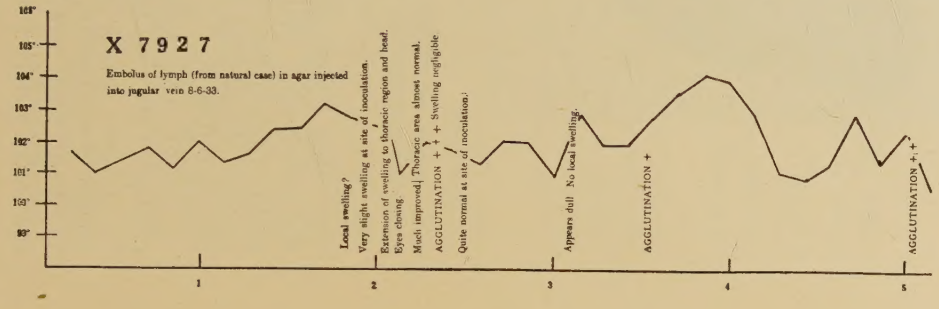
Infection with Culture from Shoulder Tumours.

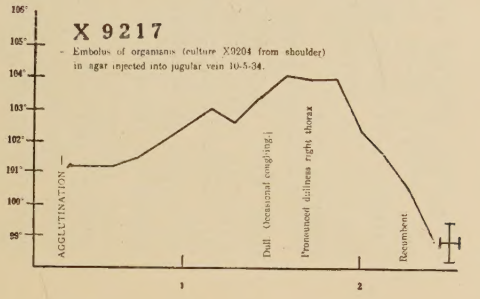
Two separate attempts were made to infect animals with first generation cultures derived from shoulder tumours. In the first experiment X8123 and X8471 were inoculated with a culture recovered from X8234 during a reaction to subcutaneous inoculation with filtered "shoulder lymph." Neither of these animals reacted to the original inoculation and both reacted severely five months later to subcutaneous inoculation with thoracic lymph from a natural case.

X7535 and X8118 were similarly inoculated with the same culture from X8234. Neither reacted and both were negative when examined *post-mortem*. At the beginning of the experiment the sera from all four animals gave negative results to the agglutination test, but from the 77th day onwards all gave positive reactions, three of them very strong positives. It will be observed that in spite of these positive reactions X8123 and X8471 were not protected against a subcutaneous inoculation of virulent lymph, and X7535 and X8118 were not found to be infected when examined *post-mortem*.


X 6881 CONTACT INFECTION
Contact begins.
AGGLUTINATION –
Contact ends
AGGLUTINATION –
AGGLUTINATION –
AGGLUTINATION –
Appears dull. Muzzle dry
Still dull.
Appears sick. Lying down
Still sick. Dullness right thorax
Lying down.
AGGLUTINATION + + +
EXPOSURE 10 DAYS.
INCUBATION 27 DAYS
1
2
3
4
5
6
7
8


X 5950 CONTACT INFECTION
Contact begins
AGGLUTINATION –
Contact ends
AGGLUTINATION –
AGGLUTINATION –
AGGLUTINATION –
Appears normal. Feeding
AGGLUTINATION + + +
EXPOSURE 10 DAYS.
INCUBATION 30 DAYS.
1
2
3
4
5
6
7
8


X 7927
Embolus of lymph (from natural case) in agar injected into jugular vein 8-6-33.
Local swelling?
Very slight swelling at site of inoculation.
Extension of swelling to thoracic region and head.
Eyes closing
Much improved. Thoracic area almost normal.
AGGLUTINATION + + + Swelling negligible
Quite normal at site of inoculation.
Appears dull. No local swelling.
AGGLUTINATION +
AGGLUTINATION + +
1
2
3
4
5


X 9217
Embolus of organisms (culture X9204 from shoulder) in agar injected into jugular vein 10-5-34.
AGGLUTINATION –
Dull. Occasional coughing.
Pronounced dullness right thorax
Recumbent
1
2

In the second experiment three animals, Nos. X8619, X9201 and X9217, were inoculated intrajugularly with first generation culture recovered from a shoulder tumour in X9204 during a reaction to subcutaneous inoculation of thoracic lymph from a natural case.

The temperature of X9217 rose on the sixth day after inoculation and this animal developed an acute pleuro-pneumonia, affecting chiefly the right lung, and leading to death on the 17th day after inoculation. *Post-mortem* there was rather more than an ounce of exudate in the right half of the thoracic cavity. The posterior two-thirds of the right lung was much enlarged with a thick fibrinous deposit on the visceral pleura. The whole of the affected portion of the lung was in varying stages of red hepatisation. The interlobular septa and peribronchial connective tissues were thickened and distended with lymph. The left lung contained

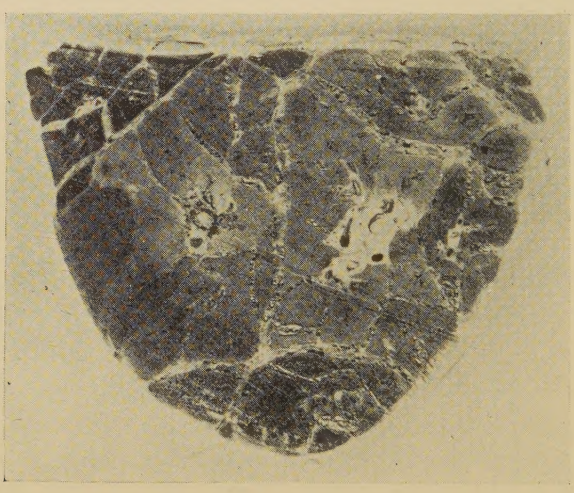


FIG. 2.
Cross section of affected portion of right lung of X9217. Acute fatal pleuro-pneumonia provoked by an embolus containing culture derived from a shoulder tumour.

four large lesions and eight small ones. The large lesions were not encapsuled and macroscopically they were typical of acute pleuro-pneumonia (Fig. 2). Histologically the lesions were equally diagnostic and a pure culture was recovered from the thoracic lymph.

X9201 also commenced to react from the sixth day, but clinically the reaction was not so severe, although extensive areas of dullness could be detected in the right lung and the animal coughed frequently. The animal lost condition but made a partial recovery from the attack. On the 27th day X9201 gave a markedly positive reaction to the agglutination test, and on the

32nd day, roughly twelve days after the acute reaction had subsided, X9201 was destroyed for examination. *Post-mortem* the right lung was found to contain two large areas together roughly the volume of a cocoanut, which were evidently in process of encapsulation. In one of these lesions the tissue was breaking down to a brown grumous fluid. In the left lung there were four sequestra, the largest roughly the size of a cricket ball and the remainder about the size of a walnut. These sequestra were well separated from the surrounding normal tissue. Pure cultures of the organism were obtained from the softened lesion in the right lung both before and after filtration. Histologically these lesions were typical.

The remaining animal, X8619, never reacted to the inoculation, although from the fourth to the seventh weeks he gave strongly positive reactions to the agglutination test. He was destroyed on the 42nd day for examination and proved to be unaffected.

INFECTION BY CONTACT.

Seven cattle, Nos. X7878, 7807, 7795, 7909, 7819, 7911 and 7769, were put in contact in a large loose-box with three cattle chronically affected with pleuro-pneumonia. The exposure lasted ten-and-a-half months; and neither the naturally affected animals nor any of the in-contact animals showed any definite clinical reaction during this period, except that the naturally affected animals were occasionally observed to cough.

All the animals in the experiment were periodically tested by the agglutinin-precipitin test of Dahmen, and/or the ordinary tube agglutination test, using as antigen in the latter test a carbol-saline emulsion of the organism prepared by centrifuging a broth culture at 11,000 revolutions for 15 minutes and taking up the precipitate in carbol-saline.

Nos. X7878, 7807 and 7795 remained negative to these tests throughout the experiment and were eventually discharged. Nos. 7909 and 7819 remained negative throughout and were examined *post-mortem* with negative result. X7911 was at first negative to the blood tests, but gradually from giving doubtful results with occasional positives in the earlier months, became completely positive in the eighth month, and was destroyed. On *post-mortem* examination X7911 was found to have a small sequestrum in the left lung, roughly $\frac{3}{4}$-in. in diameter. The sequestrum contained caseous material, and the surrounding tissue for about $\frac{1}{3}$-in. in radius was congested and in parts hepatised, with distended septa. Histologically the tissue changes surrounding the sequestrum were typical of the disease.

X7769, at first negative to blood tests, became strongly positive by the fifth month. This animal was destroyed after five-and-a-half months' contact. *Post-mortem* an adhesion was

found towards the lower border of the right lung and beneath it a sequestrum about 1½-in. in diameter containing brownish yellow material of the consistency of thick cream. The lung tissue for a radius of 1½-in. to 2-in. around the sequestrum was congested and there was marked infiltration of the septa. This area was not completely hepatised, but histologically the picture was a typical one. Attempts to obtain cultures failed, and two cattle inoculated subcutaneously with ground up tissue also failed to react, though they were later proved to be susceptible.

In this experiment then, two animals out of seven exposed to infection by contact with chronic cases of pleuro-pneumonia became infected.

An attempt was next made to secure infection under more closely controlled conditions, and here it was fortunate that we were able to obtain acute cases of the disease as a source of infection. Five cattle, Nos. X7365, X6574, X6025, X5950 and X6881, were placed in contact with four acutely affected animals obtained from Kajiado. The exposure lasted ten days, by which time all the naturally affected animals were dead.

Two of these animals, Nos. X7365 and X6574, remained negative to blood tests and clinically healthy for prolonged periods after the ten days' exposure, and each reacted severely to a subcutaneous inoculation of lymph nine months after the beginning of the experiment.

A third animal, X6,025, was never observed to react clinically but became positive to the agglutinin-precipitin and agglutination tests on the 19th day, and remained so until he was destroyed on the 106th day. The *post-mortem* examination gave a completely negative result.

X5950 gave a marked temperature reaction 30 days after the finish of the ten days' exposure. During this reaction, which lasted nine days, no lung changes could be detected by clinical examination. On the 29th day, *i.e.*, the day before the temperature reaction commenced, this animal's blood was negative to the agglutination and precipitin-agglutinin tests. Thirteen days later he gave a strongly positive result to both tests and his blood remained strongly positive up to the day of his death. He was destroyed on the 121st day and at *post-mortem* examination the left lung was found to be firmly adherent to the chest wall over an area approximately 12-in. × 9-in. in extent. In the cardiac lobe of the left lung there was a well isolated sequestrum roughly the size of a cricket ball. Macroscopically and microscopically the lesion was diagnostic, but attempts to obtain cultures from the lesion were unsuccessful.

X6881 reacted on the 27th day after the close of the period of contact. The temperature reaction lasted 17 days. Dullness could be detected over a large area of the right lung during the second week. On the 29th day, *i.e.*, two days after the commencement

of the reaction, the blood of X6881 was negative to the agglutination and precipitin-agglutinin tests, but on the 42nd day it was strongly positive and remained so until death. This animal was destroyed on the 136th day.

Post-mortem the thoracic cavity contained no fluid but there was an adhesion of the right lung to the chest wall, roughly 2-in. × 1-in. in extent. The visceral pleura of the right lung was markedly thickened, tough and opaque; while the parenchyma of the lung appeared normal, the lobules were widely separated by septa which were composed of loose areolar fibrous tissue partially organised. There was one small encapsuled sequestrum roughly the size of a haricot bean. The left lung was normal. The appearance of the right lung and pleura indicated that there had been resolution of an acute pleuro-pneumonia that had involved the whole organ.

DISCUSSION.

It has been possible for the first time to reproduce by inoculation of the organism, either in lymph or pure culture, the pathological condition regarded as characteristic of contagious bovine pleuro-pneumonia. Infections have been obtained once in many attempts by the intratracheal route, and five times in nine attempts by embolus production. The evidence in support of the general assumption of a causal relationship between the organism and the disease is now almost complete, lacking only a demonstration of the contagious character of the disease set up by inoculation. Unfortunately in these preliminary experiments none of the animals that have been kept in contact with the inoculated animals have contracted the disease. In view, however, of the experience already gained in the study of infection by contact, there is no reason to anticipate that the demonstration of contact infection from inoculated animals will offer any very great difficulty if the disease produced by inoculation is actually contagious bovine pleuro-pneumonia.

The technique devised by Tuttle for the production of emboli in the lungs is likely to prove of the greatest value in future investigation of the disease. The high proportion of infections already obtained by this technique suggests that it will be advantageous to use the method in investigations of the immunising power of different vaccines and the duration of immunity in vaccinated animals, in place of the more artificial method of subcutaneous inoculation.

Still more encouraging is the opportunity it will afford to investigate the persistence of infection in partially or clinically recovered animals, where the lesions are in process of sequestration. One of the greatest difficulties in the control of the disease by vaccination and segregation of in-contacts is to decide when a quarantine may safely be lifted; and, although there are occa-

sional records of the recovery of the organism from natural cases after prolonged periods (even up to two years), it has not previously been possible to obtain perfectly authenticated information on this point.

The most interesting feature of the infections obtained by contact is the length of the period of incubation after all contact has ceased. In the two animals for which data are available this period was 30 and 27 days, which is definitely longer than the period formerly allowed in Kenya as an incubation period, when control by the conglutination test was applied to animals exported from certain infected native reserves.

A number of serological tests has been devised to detect the "lunger" or apparently healthy but infected animal, but when these tests have been applied to the detection of carriers among animals exposed to infection the results have been largely disappointing.

Apart from general difficulties in the technique of the tests, which may be ascribed in great part to rapid changes in the virulence of the organism in serial culture, apparent failure to detect all the infected animals in an outbreak may in certain cases be explained by the length of the incubation period. Failures in the opposite sense, *i.e.*, the obtaining of repeated positive reactions from animals that are found *post-mortem* to be completely healthy, are equally explicable by the fact noted in this paper that an animal exposed to infection (either by contact or inoculation) may develop antibodies in the absence of recognisable lesions and even without acquiring any demonstrable immunity. Such an animal may be a strong reactor to any serological test. In a few cases it will be observed that animals have recovered from severe subcutaneous reactions to lymph or have completely resisted such inoculations and yet have afterwards failed to react to the usual serological tests. This, however, may not be a matter of very great importance in control of the actual disease.

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NOTES ON THE HUMAN TRYPANOSOMIASIS OF SOUTHERN RHODESIA.

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In November, 1909, there arrived in Hartley from North-Eastern Rhodesia a European named Walter Armstrong suffering from a fever which did not yield to treatment. Blood preparations were taken by Dr. Alexander Mackenzie, who found a trypanosome in them. Smears were sent to me and I also found trypanosomes. The patient was then sent to Salisbury and I obtained, through the courtesy of the late Dr. Heygate Ellis, a quantity of blood with which I originated a strain of trypanosomiasis in laboratory and domestic animals. From the first I was struck by certain peculiarities of the infection set up which did not conform with standard descriptions of that caused by *T. gambiense,* and this led me to contribute to the *Journal of Comparative Pathology and Therapeutics,* June, 1910, a " Note on the passage of a human trypanosome through domestic animals."[1] In this note which left my laboratory before the end of March, 1910, I drew attention to the susceptibility of rats, guinea-pigs, rabbits, sheep and a mule to artificial infection with this human parasite, and pointed out the fact that these animals, thus infected, suffered from a more severe and acute sickness than similar animals inoculated with other trypanosomes. In a later contribution to the *Journal of Tropical Medicine and Hygiene,* January 16th, 1911,[2] I again emphasised the extraordinary virulence of the disease set up in domestic animals and gave the periods of incubation and duration of the disease as follows:—

Animal.	*Average Period of Incubation.*	*Average Duration of Disease.*
Rabbit	7 days	24 days
Guinea-pig	8 „	38 „
Rat	5½ „	36 „
Mule	6 „	100 „ (about)
Sheep	6 „	47 „

I also wrote " In rabbits and sheep there is a remarkable œdema of the face, especially that part situated between the eyes and nostrils . . . . In those sheep which have shown no other symptoms save the irregular temperature, and where trypanosomes have not been found in the peripheral blood, the swelling of

the head has enabled me to recognise the infection." I also pointed out the resistance of the parasite to atoxyl.

In February, 1910, Walter Armstrong was sent to Liverpool, and Professor Stephens while examining in class work a stained specimen of rat's blood containing what was supposed to be *Trypanosoma gambiense*—that is, the trypanosome of ordinary sleeping-sickness—noticed a certain peculiarity in the morphology, namely, the fact that in some parasites the nucleus was placed far back in the body of the organism. This feature had been observed by me, and indeed had been depicted in the drawings accompanying my article to the *Journal of Comparative Pathology and Therapeutics*, but I had not regarded it as of particular importance, thinking it to be one of the many unusual forms commonly met with in blood smears where the parasites are disintegrating or are changed during the preparation of the specimen. Stephens and Fantham, however, regarded this characteristic as evidence of a new species and contributed a note to the *Proceedings of the Royal Society*[3] " On the peculiar morphology of a trypanosome from a case of sleeping-sickness and the possibility of its being a new species," to which they gave the name *Trypanosoma rhodesiense*. Considerable controversy arose as to whether they were justified in creating a new species on such slender evidence, and Yorke and Blacklock drew attention to a similar feature in a strain of *T. equiperdum*, while Wenyon still further discounted the value of this irregularity for diagnostic purposes by showing the posterior nuclear arrangement in a strain of trypanosome thought to be *T. brucei* obtained from animals in the Bahr-el-Ghazal. Bruce also found similar forms in his Zululand preparations of *T. brucei* taken in 1894, and in 1924 I received from Mr. W. Robertson, Acting Director to the Veterinary Research Laboratory, Onderstepoort, that which I had desired to obtain from the first, namely a strain of *T. brucei* derived from a natural case of " Nagana " in a mule in Somkele, Zululand, and found in sub-inoculated animals numerous trypanosomes having the morphological peculiarities regarded as specific for *T. rhodesiense*.

Now, sleeping-sickness had for some time been known to exist in Northern Rhodesia along certain parts of the Luapula River, and on the shores of Lake Meru, in the Kaluwisi Valley, and on the shores of Lake Tanganyika. Medical officers had been working in those districts for some years and all known cases of the disease had been segregated in special hospitals or camps. In these areas the *Glossina palpalis* was known to be present and until 1909 this was the only species of tsetse-fly known to be capable of transmitting human trypanosomiasis in Africa. It is true that in 1908 Kinghorn and Montgomery had written ". . . . at present all other species must be regarded with equal suspicion " and[4] later, in a Second Report on Human Trypanosomiasis in

Northern Rhodesia, they expressed the opinion that *Glossina morsitans* was dangerous under all circumstances. Also, in 1909, Dr. Hearsey published in the "Nyasaland Sleeping-Sickness Diary" an account of six cases of Sleeping-Sickness in persons who had not visited a *palpalis* area. One was a European infected in Nyasaland or Northern Rhodesia—perhaps in the Luangwa Valley, two cases probably contracted the disease in Nyasaland or Portuguese East Africa and the others were natives who also may have become infected in Nyasaland or Northern Rhodesia. The arrival, therefore, of Walter Armstrong in Southern Rhodesia from Kasama in North-Eastern Rhodesia, *via* Mzaza, the Luangwa Valley and Beira, with trypanosomes having peculiar characteristics and exceptional virulence to domestic animals was particularly alarming, inasmuch as he had never been in a *palpalis* infested country.

About the same time, Dr. May, Principal Medical Officer for Northern Rhodesia, in an Official Report[5] of a tour through the sleeping-sickness areas of that territory, gave an account of eight cases of sleeping-sickness. In some of these cases it seemed certain that the subjects had not been in contact with *palpalis* and must have visited the Luangwa Valley. Later reports recorded the finding of two other cases near Nawalia on the Nyamadzi River, in natives who had never travelled, and five other cases—three of them women who had never travelled—in the Luangwa Valley below Hargreaves. My records thereafter are somewhat incomplete, but between December, 1909, and December, 1911, 29 cases, of which four were Europeans, were reported in North-Eastern Rhodesia, and the Luangwa Valley was definitely regarded as a focus of infection. Careful research was made by Neaves and others in the Luangwa Valley for *palpalis* but without success, and the opinion was expressed that it was improbable that that species could exist south of the Zambesi-Congo watershed. Hargreaves, the most important focus of infection, was more than 200 miles from a *palpalis* area.

The first notification of the presence of sleeping-sickness or trypanosomiasis in Southern Rhodesia—to quote Dr. Fleming,[6] the late Medical Director—was received in a cable from the London Office of the British South Africa Company on August 1st, 1912, which stated that H. R. T., an official who had been stationed in the Sebungwe District, and who was then in England on sick leave, had been examined at the London School of Tropical Medicine and found to be suffering from trypanosomiasis. Enquiries were made as to the official's movements previous to leaving England, and it was found that he had *never been north of the Zambesi* or in any known sleeping-sickness area, or come in contact with tsetse-fly except in the Sebungwe area, the presumption being that he contracted the disease there. Investigations were therefore carried out by Drs. Fleming, Stohr

and Huggins (our present Prime Minister) and Messrs. Carbutt (of the Native Department) with Jack as Entomologist, who visited the Sebungwe early in October and travelled through the fly area, visiting every native village and examining every individual who could be found—men, women and children. Eleven infected natives were detected. The report states that the natives in the fly-belt had no cattle, few dogs, and only small herds of sheep and goats. Examinations of these were made, and "a certain percentage were found to be infected with trypanosomiasis." Successful inoculations of rabbits were made with a human strain, a dog strain, and a goat strain, and these rabbits were sent to me at Salisbury. These trypanosomes resembled those originating from Walter Armstrong and in a preliminary report I stated "Trypanosomes of the above strains with one exception correspond in motility, morphology and periods of incubation in sub-inoculated animals, and give rise to diseases clinically identical and running the same course. Appropriately stained they show the same arrangement of granules, and in each strain trypanosomes have been encountered with nucleus in the posterior third of the parasites. A trypanosome met with in two waterbuck shot in the infected area is indistinguishable from the above."[7] I pointed out that no distinction could be drawn between the trypanosomes from the latter and those met with in infected natives and domestic animals in the Busi Valley. They were long forms of the *T. brucei* type, and many showed the posterior nucleus previously referred to. Whatever this posterior nucleated trypanosome was, it was found to exist in man and animals—domestic and wild, in Southern Rhodesia, where *palpalis* did not exist but where *G. morsitans* only was known to be present, and since at that time this fly was known to exist in over 10,000 square miles of territory the situation appeared somewhat alarming.

In 1911 a Commission was sent to Nyasaland by the Colonial Office, under the direction of the Royal Society, to enquire into Nyasaland Sleeping-Sickness. Sir David Bruce was in charge of this Commission and in a letter to me from Kasu Hill, Angoniland, Nyasaland, dated August 5th, 1912, he wrote: "I have been here since January working principally at the human trypanosome disease of Nyasaland. This is called *Kaodzera* by the natives and seems to be caused by *T. rhodesiense*. I cannot find any long dimorphic forms (Nagana type) in the wild *G. morsitans* or in the antelope round here except this posterior nucleated *T. rhodesiense*. Have you a nagana type in Rhodesia and, if so, is it *T. rhodesiense*, or what?" The Commission came to the conclusion that Nyasaland sleeping-sickness caused by a posterior nucleated trypanosome was a separate and distinct disease from the Congo sleeping-sickness. It was soon struck by the fact that although the fly country of Nyasaland extended almost interruptedly into the Zululand fly country and cases of nagana were

reported from Portuguese East Africa, German East Africa, everywhere, in the wild tsetse-flies and the wild game it was always *Trypanosoma rhodesiense* which was present. To use Bruce's own words " It then began to dawn upon the mind of the Commission that perhaps *Trypanosoma rhodesiense* was in reality the old nagana parasite masquerading under a new name. As soon as possible a strain of the latter was produced from Zululand for purposes of comparison, and the Commission, after a good deal of work, came to the conclusion that the *nagana* parasite and *Trypanosoma rhodesiense* are in reality one and the same. They are identical in morphology, in their action on animals, and in their manner of development in the tsetse-fly, and until further proof is brought forward that they are separate species the Commission decided to consider them identical."[8] Bruce then added " This is a very important matter, because if *Trypanosoma brucei* and *Trypanosoma rhodesiense* are identical, then we may expect to find cases of ' Nyasaland sleeping-sickness ' cropping up in all parts of Africa where nagana occurs—in the Sudan, Uganda, British East Africa, North-Eastern Transvaal and Zululand." " If, on the other hand, they are separate species, then cases of this disease would only be expected to occur where *Trypanosoma rhodesiense* is found in addition to nagana. In the past nagana has always been regarded as harmless to man, and certainly no harm seemed to come to those who lived or travelled in " fly country."

Kinghorn and Yorke in 1912[9] found that trypanosomes were of frequent occurrence in the domestic stock of North-Eastern Rhodesia and also in the big game, and a conservative estimate placed the number infected at about 50 per cent. In an examination of 127 wild animals, mostly antelope, from 58 of which inoculations were made, they isolated what they believed to be the human trypanosome from eight antelope and a wart-hog. The trypanosomes obtained were compared with a strain isolated directly from man, and also with a strain from naturally-infected flies, and the three appeared to be identical. The posterior nucleus was met with in them all. In the Luangwa *T. brucei vel rhodesiense* was discovered in 16 per cent. of game examined. These observations also proved that this trypanosome was conveyed by *G. morsitans* and that the percentage of wild flies infected varied with the altitude. In a valley (2,100 feet, temperature 75° to 83° F.) 1 in 534 flies was found naturally infected, whereas on the plateau (4,400 feet) with a mean temperature 15 to 20° F. lower than in the valley, only 1 in 1,260 was found infective. Flies fed and kept at the lower temperature did not become infective, though it was shown that after a period of 60 days at the lower temperature the flies became infective when the temperature was raised. Bruce and his co-workers in Nyasaland found also that *G. morsitans* was the principal agent of transmission but that

G. pallidipes was also concerned. The development in the fly was identical with that of *T. gambiense* and the animal strains of *T. brucei.*

In certain correspondence published in the *Veterinary Journal* at the time, I protested against the coining of the name *T. rhodesiense,* not only because I did not consider there was sufficient evidence that the trypanosome was a species *sui generis* but because I considered the name might be harmful to this country which in those days, even as now, was in need of white population. It seemed undesirable that the country should be labelled as the home of a very virulent type of sleeping-sickness. And in view of the fact that from that day to this only seven European and 49 native cases have been detected in Southern Rhodesia, and the identity of the so-called *T. rhodesiense* is still under dispute, I feel that my protest was justified. I find that I pointed out that if the trypanosome was so frequently present in game and was commonly transmitted by the *G. morsitans* which was widely distributed in large numbers, it was surprising that the disease was so rare and so strictly localised. One would have expected it to spread as a veritable plague throughout the Luangwa Valley and possibly through other parts of the country, instead of showing a local distribution and tendency to disappear. In the laboratory the virus had to be treated with considerable respect, yet natives whose culinary arrangements and table manners were somewhat crude could apparently wallow in the blood of game slaughtered for food and eat the uncooked flesh with impunity. Dr. May in a report on Sleeping Sickness in Northern Rhodesia in February, 1912, pointed out that ". . . . the great majority of the cases of human trypanosomiasis found in or attributable to the Luangwa closed area have been on or close to four main routes." He emphasised the fact that ". . . . a preponderance of the disease had up to the present been found on, or in proximity to, the main traffic routes from a known *gambiense* endemic area is a point in favour of a close relationship between the two infections. The fact that there is a different intermediate host and certain differences in morphology and virulence, can possibly be accounted for by the adaptation of the organism to its surroundings." This did not indicate that the game were the source of virus unless the virus had first become accommodated to the game from infected man. Infected buck, tsetse-flies and native villages were by no means confined to such routes.

These circumstances gave rise to some suspicion that the so-called *T. rhodesiense* might not be derived from *T. brucei* but merely *T. gambiense* modified by transmission through *G. morsitans* rather than *G. palpalis,* its usual vector. Sanderson[10] pointed out that ". . . . where *palpalis* is the definite host, large numbers of cases occur in individual villages, and eventually all the inhabitants become infected. The migratory habits of

morsitans, on the other hand, together with the lack of a constant place of resort, such as the watering place of a village, give the fly comparatively little chance of fulfilling its rôle of vector, and therefore the cases tend to occur sporadically. "Moreover," he added, "the Nyasaland form of the disease is so virulent that an infected person is too quickly incapacitated to multiply the number of infected fly by acting as carrier to any appreciable extent."

Kleine and Taute, working in German East Africa, believed the human cases in those areas to be due to a distinct trypanosome and not *T. brucei*. Taute[11] having established a strain of that parasite in a dog by inoculating it with blood from many infected wild animals, and having waited until characteristic posterior-nucleated forms appeared, deliberately inoculated himself with large quantities of the blood of the dog and remained healthy. He also allowed to feed upon himself *G. morsitans* previously and subsequently shown to be infective, and yet he was in perfect health when he visited me as he passed through Salisbury in 1914 on his way to Germany. After the war he and Huber[12] inoculated 129 African natives of different tribes, and also themselves with *T. brucei* derived from four horses and two mules naturally infected. In no case did an infection result. Corson[13] more recently fed *G. morsitans* on a cow infected with *T. brucei* and then upon a guinea-pig and himself. The guinea-pig became infected but he did not. Later he fed the flies upon Smith and a guinea-pig and again the guinea-pig only became infected. He also sought to ascertain whether *T. rhodesiense* could become *T. brucei* by passage through animals. He obtained a strain of *T. rhodesiense* from a native Bupamba and for 19 months passed it through sheep and then by *G. morsitans* and *pallidipes* to guinea-pigs. From one infected guinea-pig he inoculated himself and became infected with posterior-nucleated parasites in his blood.

Thus it would appear that in vast areas of central East Africa where *G. morsitans* is the predominating tsetse-fly the game harbour a posterior-nucleated parasite which may occasionally become infective to man. As pointed out by Bruce in 1911, if the parasite is *T. brucei* one might expect the infection of man to be more common, but time has shown that it is comparatively rare. If, therefore, the infecting organism is *T. brucei*, what are the unusual circumstances which render it infective to man? I have mentioned Sanderson's theory which attributes the sporadic appearance of cases to the migratory habits of *morsitans*.

Warrington Yorke, Adams and Murgatroyd[14] demonstrated that when human blood, plasma or serum is added to animal-derived trypanosomes, *T. brucei vel rhodesiense* and *T. congolense*, they are rendered non-infective, whereas *T. gambiense* remains unharmed by the serum and still infective. But even

T. gambiense after many passages through animals may lose its resistance to human serum. The same investigators enumerated the following hypothesis :—

"*T. gambiense* is, like *T. rhodesiense,* identical with *T. brucei,* its apparent greater differences being merely due to more profound modification resulting from numerous passages through the human host. In other words, the source of both trypanosomes pathogenic to man—*T. gambiense* and *T. rhodesiense*—is *T. brucei,* of which the natural reservoir is the game. The game trypanosome is not pathogenic to normal man because of the protective trypanocidal action of his blood. Under certain conditions (pathogenic and dietetic) the trypanocidal substance disappears from the blood of man and he becomes susceptible to infection with the game trypanosome. If such an individual becomes infected he develops a *rhodesiense* infection. These infections are seen typically in *morsitans* regions where the chief food supply of the fly is game or stock; they are of sporadic occurrence and are unlikely to assume the form of considerable epidemics." But one is tempted to enquire, " Why not? " Once *T. brucei* becomes accommodated or adapted to man why should not the infection be readily transmitted by *morsitans,* and if *morsitans* is plentiful why should not the disease assume the form of an epidemic? Generally speaking, however, it does not do so. Medical surveys have shown that it certainly does not do so in this country. If *morsitans* so readily transmits *T. brucei* to animals why not to man? Hamerton, a co-worker with Bruce, has recently suggested that the accommodation of *T. brucei* to human serum may take place in the fly. As we know, trypanosomes may become resistant to drugs (drug fast), and similarly resistant to serum (serum fast). The tsetse-fly may live several months and requires a feed of blood every few days. Hamerton[15] suggests that experiments should be carried out " with a view to ascertaining whether the nourishing of the game-derived-trypanosomes, partly or wholly, on human blood during the prolonged cycle of their development in the body of the insect host, is an influence in adapting *T. brucei,* and possibly animal trypanosomes, to life in the human body." He adds: " I submit, we have reason to believe that the large African fauna may act as a buffer state in absorbing the attacks of the tsetse-fly population and that, by confining the transmission of their trypanosome parasite to their natural vertebrate hosts the big game are a means of holding up the fly and preventing its dispersal through the haunts of man and the dissemination of trypanosomes among the human population." If Hamerton's theory is correct, then infected man, not game, is the chief danger to man, and it may be necessary to remove him from the danger zones.

Let us now return to the consideration of human trypanosomiasis in Southern Rhodesia. Since 1912 to date there have,

according to the information given me by the Medical Director, been only seven European cases, all of which have contracted the disease in the Sebungwe and West Hartley districts. No cases have been recorded from the Lomagondi and Darwin districts, in parts of which *G. morsitans* are plentiful and where to my own knowledge domestic animals become infected with a *brucei* type of trypanosome. Four of the seven cases proved fatal. During the same period some 49 native cases have been recorded and no doubt there have been others. But nothing of the nature of an epidemic has occurred.

During the last two years some alarm has been caused by the death from trypanosomiasis of a European, Mr. L—, who had been on a shooting trip in the Umniati area. During the course of his trip he is said to have visited Gowe, a fact of importance as will be seen later. It is said that the period of incubation in this case was less than a fortnight, which goes to incriminate this area. Another European, Mr. A—, working at the Ivy Mine near Robb's Drift (about 18 miles from Gowe) also developed the disease. In February, 1933, a native hunter contracted the disease, and about the same time as Mr. L— two other cases, employed by the Native Department as hunters in the West Hartley area, died from trypanosomiasis. Examination of some 120 other native hunters also revealed three mild cases which responded to treatment. In addition, a native servant of a police officer who went on patrol to Gowe developed and died of trypanosomiasis. Two other native cases occurred on a farm in Lomagondi, some 30 miles from the nearest fly area. The origin of their infection has not, as far as I know, been determined.

On July 5th, 1934, Dr. Blair, one of the officers of the Medical Department Trypanosomiasis Survey, brought to my laboratory an old native named "Kahondera," the headman of a village at Gowe on the Sebungwe (left) bank of the Umniati River, 18 miles north of Robb's Drift. He is said to have lived his whole life in "fly" country but to have visited Gokwe, Gatooma, Gambesa's and the Copper Queen Mine. The old man was thin and suffered from bronchitis but did not exhibit any marked symptoms of trypanosomiasis. On examination of his blood I found some *Filaria perstans*, one malaria parasite, and one trypanosome in 500 fields examined under an oil-immersion lens. Dr. Blair and Dr. Mackenzie had previously found trypanosomes in his blood and glands. His blood was taken on July 5th and was inoculated into two guinea-pigs, and again on July 7th, when it was inoculated into one of the two guinea-pigs, two mice and a rabbit. The rabbit showed trypanosomes on the eighth day and died from an acute form of the disease, with characteristic swelling of the head, in 21 days. One of the two guinea-pigs, the one inoculated twice, showed trypanosomes 21 days after the first injection, or 19 days after the second, and died 119 days after the

second injection. The second guinea-pig, the one inoculated once on July 5th, did not appear to become infected. It was re-inoculated from an infected mouse on July 26th. On August 14th a single trypanosome was found in a blood smear, but no more were found until October 9th, when after immersion in cold water another trypanosome was seen. Thereafter the disease developed rapidly and the animal died unexpectedly on October 15th, 102 days after inoculation. One of the mice inoculated on July 7th showed trypanosomes on July 15th, eight days after inoculation, and died on September 4th, 59 days after. The second mouse did not appear to become infected after inoculation with Kahondera's blood, but when inoculated with blood from the first mouse on July 26th showed trypanosomes on the same day. It was found dead on August 28th, 52 days after inoculation, but this cannot be regarded as a normal case. Attempts to infect sheep from early cases did not succeed, but after the virus had become exalted through rabbits and mice it became accommodated to sheep. A ewe inoculated September 28th from a mouse showed trypanosomes on October 6th and died on November 1st. Neither this nor any other sheep inoculated with this strain showed the enlarged head characteristic of the Armstrong strain but, it may be remembered, this swelling was not present in sheep inoculated in 1913 with the Sebungwe strain. The enlargement, however, was very marked in the first rabbit and in others infected earlier with the " Kahondera " strain.

The importance of original strains in determining the virulence of a trypanosome is shown by the following table, which indicates how rapidly the virulence of a strain may become exalted by " passage " and how useless figures relating to " period of incubation " and " duration of disease " may be unless based upon the first series of inoculations from a natural host.

Date of Inoculation.	*Kahondera.*	*Result.*
7.7.34	Mouse No. 1	Died in 59 days
2.8.34	Mouse No. 4	Dying in 11 days : treated : died in 26 days
13.8.34	Mouse No. 8	Died in 14 days

It is therefore not proposed to labour this report with further figures of this nature, but it may be of interest to compare those enumerated with those from early cases of the " Armstrong " and " Sebungwe " strains.

Animal.	*Strain.*							
	Armstrong.		*Sebungwe.*				*Kahondera.*	
	Pl.	*D.D.*			*Pl.*	*D.D.*	*Pl.*	*D.D.*
Rabbit ...	7	24	Human strain Dog Goat	8 or less 8 „ 8 „	13 19 27		8	21
Guinea-pig	8	38		—			19 or 21	117 or 119
Mouse ...	—	—		—		—	8	59
Rat ...	5½	36		—		—	—	—
Mule ...	6	100 (about)		—		—	—	—
Sheep ...	6	47	Via rabbit	4		49	8 (after passage)	33

With regard to morphology, there does not appear to be any distinguishing feature in blood smears from the animals of the three strains except that in the Kahondera strain anterior-nucleated forms are occasionally met with. Such forms may have been present in the Armstrong and Sebungwe strains but were not recorded.

The case of "Kahondera" is of the utmost importance. During the year 1934 a very careful survey was organised by the Medical Department, made up of two parties, the one dealing with the Hartley-Sebungwe area, the other with the Lomagondi area. Three native cases were discovered by Dr. Blair, in charge of the former, two of which were native "carriers," namely Kahondera and his son. These cases were discovered at Gowe, and infection in the case of Mr. L-- and other native cases could be traced to this village, which is now considered to have been the focus of the recent outbreak. It is of great interest to note that three surveys carried out in 1934 with great care and patience in the Lomagondi and Darwin districts failed to find any cases or any description of cases suggesting sleeping-sickness. The Medical Director writes: "In view of this fact and of the fact that these two districts are much frequented by native immigrants and European hunters, we are justified in concluding that the large *morsitans* area in these two districts is free from infection. Further, Dr. Blair's equally careful search in 1934 in the northern Sebungwe fly region found no cases, and this area also is quite frequently visited by officers of the Native Department and the police. It is improbable that human infection has spread to that part of the Sebungwe. It therefore appears that sleeping-sickness is limited to quite a small area of the *G. morsitans* belt in the Colony and that the number of cases is small. We also feel justified in concluding that we have got hold of the focus of the recent outbreak in the neighbourhood of the Western Hartley area."

Having located Gowe as the focus of recent infection, it is interesting to note that according to the Chief Entomologist

there are certain small areas, depicted in his maps, where the tsetse-fly survived after the rinderpest in 1896. *And the Gowe area is one of them.* Another such area is the Manzituba Vlei and environs in the Sebungwe district, and it is in this vicinity that the medical expedition of 1913 found its cases. Some of these cases were suffering from the disease in an acute form, others in a sub-acute or latent form. But from Dr. Fleming's report it would appear that there had been a good deal of coming and going from one part of the Busi area to another, and it is probable that " carriers " were localised and that even local natives visiting them or their kraals became infected and developed the disease in an acute from—just as local natives visiting Kahondera's kraal did.

In an appendix to Dr. W. Yorke's paper[16] on " The Relationship of the Big Game of Africa to the spread of Sleeping-Sickness," written in 1913, Marshall (now Sir Guy Marshall) wrote ". . . . the incidence of human trypanosomiasis in Nyasaland and Rhodesia presents a very striking similarity with that which we find in West Africa, and, in conjunction with other considerations, strongly suggests that we are dealing, not with a new disease, but with one which has already been endemic for some time and has merely escaped detection, as in West Africa. If this be a sound conclusion we must recognise the probability that the immune natives may themselves be reservoirs of the trypanosome, in which case Dr. Yorke's assumption that the removal of the game must necessarily eradicate the disease may prove entirely fallacious."

In my evidence before the " Departmental Committee on Sleeping-Sickness," October 1913,[17] I criticised this theory on the grounds that natural immunity against protozoan diseases is largely dependent upon reinfection, whereas after rinderpest in 1896 the tsetse disappeared for many years and in all probability any inherited immunity against trypanosomiasis died out. Just as in the case of piroplasmosis of cattle, animals exposed to infection by ticks from the time of their birth become immune, or rather premune or tolerant, and carriers, but those born on tick-free farms do not become infected and acquire no immunity. Even animals infected as calves which have for some time been running on tick-free farms lose the infection and the premunity dies out. It was thought, therefore, that any immunity in the indigenous natives against trypanosomiasis would die out in the absence of the tsetse-fly and they would neither be immune nor carriers. Moreover, in 1913 I carried out certain experiments which indicated that the young of infected mothers were not infected *in utero* or from the milk of the mother after birth. Nor did such young derive any immunity from their mothers. Indeed, it was possible to infect them with the blood of their infected mothers. Recently, I have repeated these experiments

with the same results. It would therefore appear improbable that local natives inherit any immunity. But experiments in hand suggest that premunity may be acquired by the young as the result of infection, and it is probable that native " carriers " are created not born, and derive their premunity as the result of early infection by the tsetse-fly.

As we have said before, there were certain comparatively small areas as depicted in the Entomologist's maps accompanying his report on " The Tsetse-Fly Problem in Southern Rhodesia," Bulletin 892, May, 1933,[18] where the tsetse-fly persisted after the rinderpest epizoötic of 1896, as, for example, Gowe and Manzituba Vlei and environs in the Sebungwe district. Here the local natives would be liable to infection from birth, and any premunity would be maintained by constant reinfection. In such places, if anywhere, one would expect to find carriers, and here the Commission of 1913 and Dr. Blair in 1934 actually found them. Other parts of the country appear to be free from human trypanosomiasis, and it is probable that with the clearing up of these comparatively limited areas this undesirable menace will soon be entirely eliminated from Southern Rhodesia.

The question, if only of academic importance, remains, what is the species of trypanosome which infects man in Southern Rhodesia and how is it perpetuated and transmitted? This cannot be determined by a study of its morphology. The fact that posterior-nucleated forms occur is not diagnostic. Artificially it can be transmitted to laboratory and domestic animals but the period of incubation and duration of disease in them offer no clue. In some it runs a chronic course, in others, after passage, it causes a most acute infection. A similar trypanosome was met with in sheep, dogs and wild animals in the Sebungwe district in the neighbourhood of kraals where human cases occurred in 1913, but on the other hand it has been met with in animals in the Lomagondi district where no human cases have been known to occur. It is undoubtedly transmitted by *G. morsitans*, but it would appear that the transference is man-fly-man rather than animal-fly-man. One has to admit that the question as to whether the parasite is *T. brucei* in man, *T. gambiense* transmitted by *G. morsitans*, or a species *sui generis* infective to man and animals has not yet been answered.

In the course of my experiments with the " Kahondera " strain one observation has been made which is of considerable practical interest, namely that the trypanosome responds to treatment with " Bayer 205," and that this drug, whether applied to infected or healthy animals, protects them against infection for a considerable time. In the case of a rabbit treated when apparently *in extremis*, rapid recovery resulted and the animal was rendered resistant to re-inoculation with a most virulent strain 122 days after. A control animal inoculated at the same time died in 23

days. Mice, rats and sheep treated with this drug have responded in the same way. In Bayer 205, therefore, we appear to possess a prophylactic as efficient against the human trypanosome of this country as it has been found to be against *T. rhodesiense* in other countries. It may be mentioned that Duke in Uganda[19] has found " that a single dose of 1 g. of Bayer 205 given intravenously will protect a man for at least 113 days from infection by tsetse carrying cyclically *T. rhodesiense*." Also Corson[20] in Tanganyika did not hesitate to infect himself and volunteers with *T. brucei* and *T. rhodesiense* of that country, relying on Bayer 205 injected at the appropriate time to arrest the disease. To a large extent, therefore, human trypanosomiasis has lost its terrors.

In the light of recent knowledge, therefore, it would appear that the evacuation of the natives from certain comparatively restricted areas to tsetse-free country, and the use of Bayer 205 as a prophylactic, should result in the early elimination of human trypanosomiasis from Southern Rhodesia.

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EXPERIMENTS ON THE VALUE OF PROTECTIVE INOCULATION AGAINST THEILERIA ANNULATA OF CATTLE.

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The experiments which are here described are a continuation of the work on the experimental transmission of theileriasis previously published by the writer (1927). In the course of this work ten imported heifers were used, and from one of these, a shorthorn heifer (No. 6), the maintenance of the various types of tick fevers was continued by the periodical inoculation of native or cross-bred calves. In the original experiments all the imported animals with the exception of No. 6 died from various types of tick-borne infection. From No. 6 passage experiments have been continued with the object of ascertaining the effect on various types of animals and also to ascertain whether any increased immunity or resistance arises from such inoculations. It is of course to be remembered that blood inoculations may convey not only theileria but other tick infections such as anaplasmosis, *P. bigeminum, B. bovis, spirochætosis* and *Trypanosoma theileri*. Of these, anaplasms have always been seen, but other types have not been observed since the use of imported calves for experimental inoculations was discontinued. This does not, however, preclude their presence. Theileria are conveyed with certainty even when only one drop of carrier blood is used (see No. 20 in Table). (A count of the blood used for inoculation showed 400 theileria per drop.) After the preliminary incubation period theileria were found in the blood of inoculated cattle with considerable regularity and the animals remained carriers.

The accompanying table shows briefly the inoculations performed and the results.

An examination of the results indicates that inoculation methods of obtaining immunity are not very favourable. Only one animal, No. 10, remained in good health until the final field test. On free exposure to tick infection this animal died after three weeks from an acute attack of *Babesia bovis*. No. 15, which had previously passed through a very acute attack of theileriasis, succumbed from that disease within three months' exposure, in spite of being largely of native stock. Nos. 9 and 11 were destroyed on account of their condition and No. A4 died of anaplasmosis though a native animal. Nos. 20, 21 and 23 did very badly as calves after inoculation. The first two animals have

No.	*Breed of Animal.*	*Age when Inoculated.*	*Date of Inoculation.*	*Donor of Blood.*	*Amount of Blood Injected.*	*Type of infection in Donor.*	*Result.*
1	Native	6 months	3.2.25	Jersey Bull	20 c.c.	Very virulent	No marked reaction. Condition remained good. Became a carrier. Died 1928. Cause not investigated.
6	Imported Shorthorn heifer	7 „	7.6.26	No. 1	5 „	Carrier	Became a carrier (no acute attacks). Died of pneumonia 27.12.28.
A3	Native	4 „	10.12.28	No. 6	5 „	„	Died 22.5.30. Cause not investigated.
9	Holland × Damascus	3 „	12.1.30	No. A3	2½ „	„	Never did well. Became blind. Destroyed 12.10.31.
10	Holland × Damascus	4 „	1.7.31	No. 9	1½ „	„	(Castrated at 3 months.) This animal was always in good health and condition. No acute attacks. It died however after three weeks' exposure to tick infection in the field from acute *Babesia bovis* infection.
A4	Native	6 „	24.6.31	No. 9	5 „	„	Died from anaplasmosis 23.7.31.
11	Holland × Damascus	3 „	13.4.32	No. 10	5 „	„	Did badly. No acute attacks. Developed panophthalmia and destroyed 25.10.32.
15	Mostly native blood	4 „	22.10.32	No. 10	½ „	„	Passed through a very acute attack February, 1933. Subsequently condition improved slowly to normal. Transferred for field test 31.7.34. Severe attack of theileria 1.10.34 and unable to stand. Died 11.10.34.
20	South Devon × Damascus × Beyrouth	6 weeks	3.11.33	No. 10	1 drop	„	No acute attacks. Did badly for 12 months. Condition now much improved.
21	South Devon × Damascus × Beyrouth	6 „	3.11.33	No. 10	20 c.c.	„	No acute attacks. Condition bad for some time but now almost normal.
23	Holland × Native cow	5 months	16.5.34	No. 10	20 „	„	Carrier state. Acute fever 4.9.34 to 21.9.34. Theileria never numerous in the blood (1 to 6 per 50 fields). Lymphatic glands enlarged and packed with blue bodies. Still debilitated.

to some extent recovered their condition but are still far from normal.

It is obvious that native cattle possess a considerable amount of resistance as in spite of being freely infected with ticks the mortality is relatively small. A certain number however do die from tick fevers, especially theileriasis, so that such immunity as they possess is merely relative and would be better described under the term " resistance " than by immunity or pre-immunity. Field experience has shown that imported cattle freely exposed to tick infection on pastures used by native cattle practically all succumb as did those which were inoculated experimentally. Damascus cattle and their crosses have also been found to be quite susceptible in the field but less so than imported European cattle.

The origin of this favourable balance of resistance in native cattle which are freely exposed to tick infection may arise from the following factors :—

(1) Heredity.
(2) Early infection as calves or infection *in utero*.
(3) Repeated reinfection by ticks.

On the whole it would seem probable that all of these factors are concerned in maintaining resistance to severe attacks, but that such resistance is liable to break down on exposure to adverse conditions, such as starvation, fatigue, change of climate, etc.

Variation in the Course of the Disease, and Microscopic Appearance of Theileria annulata.—In general, acute, sub-acute and carrier states may be recognised microscopically. In acute attacks usually more than 70 per cent. of the corpuscles are invaded and such attacks are practically always fatal. Sub-acute attacks with recognisable clinical symptoms and fever may present a corpuscular invasion of from 10-15 per 50 fields to a 20-30 per cent. invasion, whilst carriers usually show one or two theileria per 50 fields when regular examinations are carried out. The usual type of parasite seen is of the oval shape and occasional parasites may vary considerably in size. Bacillary forms and cross forms are rare, and are observed only in the acute cases. In our experience blue bodies do not occur in the blood, but in acute cases they are found in glands and internal organs.

The following exceptions, however, have been observed :—

Blue Bodies in the Blood.—These were found on one occasion in blood taken from the axillary vein of a native animal which had just been slaughtered. They were very numerous.

Heavy Infection with Bacillary Types.—In two cases, both in native animals which succumbed, the parasites observed were nearly all bacillary types and were very numerous.

Localised Theileria.—This refers to experimental animal No. 23, a cross-bred calf from a native mother by a Holland bull. This animal, which was already for some time a carrier, passed through an attack of fever lasting for eleven days, with an approxi-

mate temperature of 104° F. (103·2° F. to 105° F.). Loss of appetite and depression occurred. The pre-scapular lymphatic glands became greatly enlarged and were found on microscopical examination to be packed with blue bodies. The blood parasites, however, scarcely increased in number at any time during the attack and were never more numerous than six per 50 fields. The gland swellings subsided and the animal recovered though still cachectic after some months.

General Considerations.—Native cattle which are exposed to tick infection throughout Palestine appear to be practically all infected, though the parasites may not always be found on blood examination. On several occasions a survey of village cattle in different parts of Palestine has been made by microscopical examination of blood films and a varying percentage showing the presence of theileria in the blood have been found to exist. Adler and Ellenbogen (1934) state that in certain areas close to Jerusalem theileriasis is rare " and the opportunity for natural infection almost negligible," and on this assumption experiments were carried out to show that native cattle from these areas possess no natural immunity. This has not been our experience, and it might be pointed out that the movement of cattle is continuous in Palestine, either in search of grazing or in trade. Should tick-free districts exist, numbers of cattle from such areas would be susceptible and would naturally succumb when tick infested. Such, however, is not the case and the deaths which occur rarely in native cattle are entirely sporadic. *Hyalomma ægyptium* is commonly found on hill cattle.

Mason (1922) found theileria in Palestine cattle and in Cyprus cattle newly arrived in Egypt. Doyle (1924) also stated that " there can be no doubt that Cyprus cattle harbour the parasite when they arrive in Egypt," and are infected to the extent of 100 per cent. Seventy-seven per cent. of these cattle developed high temperatures on arrival, with an average duration of fever of 9·5 days. It would be strange if the same condition did not exist in Palestine as in Cyprus.

Appearance of Inoculated Calves.—Calves inoculated with theileria (and concomitantly with other tick fevers) have at least for a very long time a certain clinical appearance which to the trained eye differs a good deal from that of the normal healthy animal. They are inclined to be pot-bellied and the gluteal muscles are less full. The skin is somewhat hidebound and the coat is harsh and lacks lustre. There is also a lack of vitality as compared with healthy controls.

Conclusions.—The limited number of experiments described, and which are at present being continued, do not indicate that premunition is likely to be a useful method of protecting cattle against theileriasis as it exists in Palestine. It seems clear that a true immunity cannot be procured by this method.

It can scarcely be questioned that the most desirable method of controlling tick infection consists in the complete eradication of ticks by the use of proper dipping baths and isolation from cattle infected with ticks. Such herds already exist in Palestine, but this method involves a certain expense.

As previously mentioned, it has been found that imported cattle freely exposed to tick infection on pasturage used by native cattle practically all succumb, and it does not appear that the present inoculation methods will appreciably reduce this mortality. Only native cattle would seem to withstand such exposure.

The majority of dairy cattle, however, are maintained under a system of partial protection. A certain amount of washing or spraying with dip is practised and the animals are either permanently housed or allowed limited pasturage. The records of a large local insurance society show that cattle maintained more or less in these conditions sustain a $7\frac{1}{2}$ per cent. infection due to all tick fevers and a mortality of 1 per cent. of all cattle due to theileria. Although the present experiments do not show that more favourable results will be obtained by inoculation, whether as calves or not, some further work is no doubt desirable. It remains to be ascertained whether a sufficient number of healthy animals can satisfactorily survive any form of inoculation to justify such methods as against the provision of proper dipping fluid, baths, etc. Proof is also required that the apparently healthy inoculated animals are more resistant to such occasional infection and are also equal to an uninoculated animal in milk yield, condition and fertility.

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"IMMUNE" CATTLE PLAGUE ANTISERUM MANUFACTURED IN THE FIELD.

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As there appears to be considerable doubt as to the possibility of making field anti-rinderpest serum of a standard titre, I propose to give details of experiences in the manufacture of this article in the Gold Coast, where over 100,000 cattle have been immunised by using field serum with virulent blood in double inoculation. I hold the opinion that the present method of manufacture has solved the problem of standardisation and that it is possible to produce field serum which will yield constant potency and not render results variable and inconstant, as was the experience here in the earlier period of production.

Up till 1930 the cattle-producing areas of the Gold Coast suffered continually from ever-recurring outbreaks of rinderpest and the cattle were decreasing everywhere. In 1929 it was decided to immunise all the cattle of the country and eliminate cattle plague. The construction of a serum laboratory and immunisation camps throughout the country was approved. The latter were ready in early 1930 but the building of the laboratory was postponed and actually the production of " hyper-immune " antiserum did not commence until April, 1932. However, by that time many thousands of the cattle had been immunised by using antiserum purchased from Nigeria on the first herd or two and then manufacturing our own antiserum at the immunisation camps from recovered cattle.

At the commencement the method adopted was to mark all cattle which had a severe or marked visible reaction to the double inoculation, showing clinical symptoms of at least lachrymation or nasal discharge. The more marked the clinical symptoms the better the potency of the antiserum, was more or less the motto. The serum producers were bled after definite recovery, some 21 days after inoculation, and sometimes a second time a week later. Up to two and a half litres of blood were taken from a 500-lb. beast. The blood stood in bleeding jars for 24 hours, then the weight was dropped and 24 hours later the serum was decanted and ·5 per cent. carbolic acid added as a preservative. Finally, the antiserum was used as soon as possible, usually the same day and seldom later than the day following decanting, save when it was found necessary to send serum from one camp to another, but even then it was used not more than four days after manufacture. The first immunisation was carried out in Kusasi District, where rinderpest had been enzoötic for years and the cattle possessed a certain resistance, though the mortality rate in natural outbreaks

was seldom less than 65 per cent., whereas in other parts of the country casualties amounted to over 80 per cent. The cattle of this country are all unhumped animals, known as the West African Shorthorn breed, which are much more susceptible than the zebu cattle in Nigeria and French West Africa. Nigeria serum manufactured from zebu cattle caused a high mortality when used in double inoculations in the Gold Coast.

TABLE I.

IMMUNISATION RETURN FROM THE INITIAL IMMUNISATION IN KUSASI.
April, May, June and July, 1930.

Field Serum from Herd.	*Which had Reaction.*	*Used on Herd No. and No. of Cattle.*	*Percentage Deaths.*
Nigerian Lab. Serum	—	1 (548)	6·2 per cent.
„ „	—	2 (516)	12 „
„ „	—	3 (481)	8·7 „
„ „	—	4 (492)	21 „
Nigerian Lab. Serum (248 head) Field Herd 1 (200 head)			
1	Severe	5 (448)	9·4 „ (40 out of 42 being from Nigerian Lab. Serum)
2	Severe	6 (500)	1·4 per cent.
3	„	7 (506)	2·1 „
4	„	8 (503)	4·9 „
5	Marked	9 (626)	0·9 „
6	„	10 (606)	2·8 „
7 and 8	„	11 (912)	1 „
9	Mild	12 (1046)	7·4 „
10	Marked	13 (535)	2·9 „
	Total	(7720)	6·6 per cent.

Note.—Herds 1 to 4 were already infected, so the casualty figures are not a fair test of Nigerian anti-rinderpest serum.

It will be noted that the results from the field serum though variable were quite satisfactory. The rise of mortality in Herd 12 could be explained by bad weather conditions and the breakdown to gall sickness. It appeared that our method of manufacture of field serum was quite satisfactory and that an immune serum of a potent titre was being produced.

However, when operations commenced in 1931 on the western side of the country most variable results from field serum appeared. It was known that the cattle were more susceptible and this had been guarded against by increased dosage. Table II gives an excellent demonstration of the inconstancy of the titre of the field serum produced in that area.

It was early noted that the titre of the serum varied with the severity or otherwise of the reaction of the herd from which it was drawn. To attempt to standardise the titre as far as possible the following method was substituted for that of simply selecting visible reactors for serum production. Animals of a suitable size for serum production, preferably young adults, were selected and

TABLE II.

FIELD SERUM—1931.

Serum from Herd No.	*Which had Reaction.*	*Herd No. and No. of Cattle.*	*Which had Reaction.*	*Explanation.*	*Casualties.*
1	Very severe	3 (412)	Mild	Serum very potent	2·4 per cent.
1	"	4 (498)	Severe	Dose too small	8·8 "
2 and 1 ...	"	5 (600)	"	" "	5·1 "
2	"	6 (614)	"	" "	5·5 "
3	Mild	7 (554)	"	" " and serum less potent	17·5 "
4	Severe	8 (553)	"	" "	12·8 "
4	"	9 (564)	"	" "	4·6 "
5	"	10 (593)	Very severe	" "	12·1 "
6	"	11 (496)	Marked	Serum potent and dose about right	3 "
7	Very severe	12 (400)	Mild	Dose too large with such potent serum	0·25 "
8	"	13 (460)	"	" " "	0·65 "
9	Severe	14 (570)	"	" " "	0·00 "
10	Very severe	15 (579)	"	" " "	0·17 "
11	Marked	16 (566)	Marked	Serum not so potent and dose about correct	0·11 "
13	Very mild	17 (550)	Severe	Serum impotent and dose too small	21·2 "
16	Marked	18 (581)	Marked	Serum more potent, dose about correct	2·92 "
16	"	19 (519)	"	" " "	2·1 "
17	Very severe	20 (566)	Very Mild	Serum too potent for size of dose	6·2 "
17 and 18 ...	—	21 (462)	Mild	—	0·87 "
16, 17 and 18 ...	—	Kaleo No. 1 (400)	"	—	0·75 "
19	Marked	" 2 (562)	"	—	0·6 "
19 and 20 ...	—	" 3 (446)	"	—	7·4 "
21	Mild	" 4 (544)	Marked		13·2 "
Kaleo No. 1 ...	"	" 5 (604)	Severe	Dose too small	11·1 "
" 2 ...	"	" 6 } (460)	"	" "	
" 3 ...	Marked	" 6 }	Very mild	Double dose of serum cut out definite reaction	0·4 "
" 4 ...	Severe	" 6 (417)	"	Dose too large for such potent serum	0·8 "
" 5 ...	Very severe				
		Total 13,530 head.		Total death 812. Average death rate 6 per cent.	

branded numerically. The temperatures of these animals were taken every morning and if they reacted even thermally they were marked as serum makers. There are adults which react visibly but show no temperature rise. These were also marked but were not used unless there was not the required number of the former reactors. In this procedure was the beginning of noting the thermal reaction, but still the emphasis was placed on the visible reaction, and the thermal reaction was regarded as satisfactory even if the rise was slight.

Reference to the above table will show that, in spite of the modified technique, results were variable and serum secured from herds with mild reactions produced a low and unreliable titre, though dosage was much increased when such serum was used. As much as 180-200 c.c. of the latter type of serum was given as the average dose to cattle weighing not more than 4 cwt. Herds 1 and 2 were immunised with Nigerian Laboratory serum and had very severe reactions with high casualties. In 1931-32, though results were much better all round, the field serum remained inconstant and varied considerably, depending on the severity or otherwise of the general reaction of the herd from which it was taken. The position was not satisfactory and it was decided to revise the whole procedure with a view to finding out how field serum could be standardised if possible. When considering the technique of the production of hyper-immune serum, it must be noted that successful hyper-immunisation and the production of an antiserum of a constant titre depends on maintaining the anti-rinderpest potency of the serum of the hyper-immune bullocks by the repeated injection of large quantities of virulent rinderpest blood. Now, the virus producers used to hyper-immunise the serum-producing bullocks must be bled and their blood used when the thermal reaction is high, certainly not below 103°. Thus a cattle beast which has a high temperature has high virus content in its blood and an animal with a low temperature has not. Therefore it is reasonable to assume that any bovine which during the course of reaction to immunisation (even though showing visible symptoms) shows no definite thermal reaction will not have had large enough quantities of virus in its blood to stimulate profuse antibodies, whereas any bovine showing a high temperature at any period of its reaction, even if without visible clinical symptoms, must, *ipso facto,* produce quantities of antibodies sufficient to make an antiserum of high titre. On this reasoning, it was decided in the initial immunisation of Eastern Dagomba in 1932-33 to manufacture field serum only from cattle which had showed a high thermal reaction after double inoculation. This area is a riverine one, there is much trypanosomiasis, tick-borne diseases are rampant, and altogether it was an area in which, if anywhere, bad or uneven results might be expected. The area shown in Table II has better cattle and is a much better

TABLE III.

1932-33.

Herd No.	*No. of Cattle Inoculated.*	*Percentage Deaths.*	*Percentage Thermal Reactors amongst Controls.*	*Origin of Serum given.*	*Amount of Serum Manufactured.*	*Reaction in Herd.*
1	43	2·33 per cent.	70 per cent.	Pong-Tamale Laboratory	9 litres	Very marked. Visible
2	189	9·25 „	86 „	„ „	30 „	Very severe
3	504	1·01 „	50 „	„ „	40 „	—
4	207	1·45 „	30 „	„ „	13 „	Mild
5	524	0·95 „	50 „	„ „	45 „	Noticeable
6	762	2·23 „	81 „	Field serum from Herd No. 3	90 „	Marked
7	335	6·5 „	64 „	„ „ „ 4	47 „	Very severe
8	313	0·32 „	5 „	„ „ „ 5	nil	Very mild
9	513	0·58 „	35 „	„ „ „ 6	31 „	„
10	510	1·76 „	55 „	„ „ „ 6 & 7	84 „	Good
11	545	0·92 „	65 „	„ „ „ 7	92 „	„
12	266	4·5 „	70 „	„ „ „ 9	67 „	Severe
13	653	1·68 „	80 „	„ „ (and lab. serum) Herd No. 10	83 „	Good
14	160			Test Herd		
15	626	2·07 „	70 „	„ „ „ 11	95 „	Good
16	558	1·79 „	75 „	„ „ „ 11 & 12	80 „	„
17	840	3·69 „	80 „	„ „ „ 11 & 12	69 „	Very severe
18	577	1·21 „	40 „	„ „ „ 13	28 „	Mild
19	726	0·41 „	20 „	„ „ „ 13 & 14	nil	Very mild
20	498	2·61 „	40 „	„ „ „ 14 & 15	24 „	Mild
21	517	0·8 „	65 „	„ „ „ 15	63 „	Good
22	181	3·8 „	90 „	„ „ „ 16	—	—
Total	10,047	1·97 „				

livestock district in every way. In addition, the cattle of Eastern Dagomba were badly tended by their owners. Table III shows the results.

It will be seen that results were very satisfactory here. The casualties in Herd No. 7 were due to the cattle being very feeble and in bad condition, *plus* latent trypanosomiasis and red-water. It was only after considerable doubt that it was decided to inoculate them and the fact that the death-rate was only 6·5 per cent. was astonishing. Herd 12 was inoculated with field serum, some of which was known not to be very potent. The herd arrived within the confines of the camp and had to be immunised on that account, therefore field serum was made from some animals which had not had a satisfactory thermal reaction in Herd 9 in order to make up the quantity necessary to treat the former herd. Herd No. 17 for some reason for which no satisfactory explanation could be found did not react to the simultaneous inoculation of virulent blood. It reacted to the second dose of virulent blood given one week after double inoculation, by which time the full protective action of the serum had somewhat waned. These few abnormal results in my opinion emphasise that the field serum was of a reliable and practical potency, especially in the case of Herd 17.

These constant satisfactory results were undoubtedly due to the fact that the cattle selected for the production of field serum were animals that had a good thermal reaction, showing at least a rise of two degrees above normal and for at least two days, judged from the perusal of a ten days' chart of morning temperature, evening temperatures being always unreliable in this country. Temperature taking began in herds the day before or on the actual day of double inoculation. Only strong healthy adult animals were used and field serum was not prepared from any animal which had not a satisfactory thermal reaction. Thus the size of the herds inoculated at specific times had to conform to the amount of serum which could be made from good reactors and not, as before, the reverse, when the serum produced was the quantity required for a specified number of animals. Except for Herd 12, field serum was not made from any bovine whose maximum temperature on two successive days was below 102°, and as a rule all serum makers were those whose temperatures rose from 103° to 105°. Numerous visible reactors were also temperatured and it was found that many had little or no rise of temperature, particularly noted as regards older animals. The rise of temperature found to be best for the manufacture of field serum begins on the fifth or sixth day after immunisation and rises gradually until the temperature is at its height during the seventh and eighth days, thereafter gradually falling by lysis. Visible symptoms are a bad guide, as few animals thus affected showed a regular temperature chart.

This method of selection of field serum producers undoubtedly

gives a good antiserum of a constant and high potency. When tested out with laboratory serum from Pong-Tamale, the field serum manufactured thus has shown invariably a slightly higher potency titre than the latter product, which is made from hyper-immunised animals in the usual way. Many thousands of cattle have been treated with field serum since the figures shown in Table III, and all results have casualties under 2 per cent. and with young cattle a mortality of under 1 per cent. No herd since Table III has shown even a 5 per cent. mortality and that covers a period of two years. The initial immunisation has now been completed of all the cattle of the country and annual immunisation work consists in the treatment of the young stock which have grown up since the previous year. Mainly laboratory serum is now being used, though several thousands of cattle have been immunised with field serum in 1935 in the coastal plains, where an initial immunisation campaign has been in progress. The reason of the substitution of laboratory serum is simply a matter of convenience and the fact that no unit is more than a month at one camp once the initial immunisation of an area is over. The three tables given are typical of the three stages in field serum work in this country, but Table III shows the first large-scale experiment where field serum was made solely on a temperature chart basis and the size of the herds made it fit the amount of efficient serum produce and not vice versa.

The dosage has not been shown in the various tables as in this country, owing to the varying susceptibility of the cattle in different areas to rinderpest, it varies considerably. Whereas in the north-east of the Northern Territories and in the centre the dosage of field serum made as per Table III is from 50 to 80 c.c., in the north-west the dosage is from 100-150 c.c. though the cattle are smaller. Experiments have been made in bringing field serum from one area to another and testing the potency, and it has always been found that, no matter where the serum is made, the dosage for the particular area where it is used is constant, showing that the titre is in practice also constant. It is mainly due to the incidence of natural outbreaks in the past so far as evidence shows. As the work of immunising the young cattle annually proceeds there is a tendency for their resistance to increase gradually, as is evidenced by the fact that the dosage of laboratory serum now and in 1932 is decreased though the titre is the same as proved by tests on the susceptible cattle of the reserve, which have never had anti-rinderpest treatment. For zebu cattle the titre of field serum here works out at from 10-15 c.c. per 100-lb. body weight, which is much the same as the Nigerian serum in Nigeria for that particular breed of cattle.

Summary.

Field anti-rinderpest serum manufactured by the process of selecting serum makers by observing visible or severe reactions produces serum of a very varying potency, though quite good results may occur in areas where the cattle have a certain resistance to cattle plague. In susceptible districts, high casualties invariably occur from time to time. This method is empirical.

Improvement in titre is shown when attention is paid to the thermal reaction, but when visible symptoms or a severe clinical response to double inoculation is also considered a selecting factor the serum makers still produce a product which varies and may cause heavy mortality from time to time.

It is shown that field serum produced only from cattle which have shown a definite benign thermal response to immunisation is, in practice, constant in titre and yields consistently good results without high casualties in certain individual herds. Detailed figures of over 13,000 cattle treated by such serum are shown, and since then 20,000 cattle have been immunised in different parts of the country with even better results. At no time have high casualties appeared in particular herds as was the case with the two previous methods of technique.

Acknowledgments.

1. To Mr. A. Fulton, M.R.C.V.S., for carrying out the work detailed in Tables II and III. It is due to his painstaking work and careful observations regarding the cattle in question that a really reliable field serum can be produced anywhere in the country. This year he carried out immunisation in the coastal plains area, where no information was available as to the susceptibility except that natural outbreaks usually wiped out entire herds and protozoan disease was known to be enzootic. His results are even better than Table III, covering over 20,000 cattle, over 5,000 of which have been treated by field serum and the remainder of which, some 5,000 at least, are to be treated by the latter method, owing to all laboratory serum being required for the Northern Territories annual recurrent immunisation of young stock.

2. To Mr. S. Simpson, M.R.C.V.S., who was the Veterinary Officer in charge of the first immunisation camp in this Colony and who is responsible for the observations in Table I.

3. To Mr. M. K. Olympio, African Veterinary Assistant, who was the actual person in charge of the technique of the manufacture of field serum shown in Tables I and III.

Reference.

Bennett, S. C. J. (1928). *Jl. Comp. Path. & Thera.*, Vol. xli, p. 185.

THE DIAGNOSIS OF JOHNE'S DISEASE BY THE USE OF JOHNIN.

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In the literature on Johne's disease there are many references pointing to the value of allergic tests for the detection of preclinical cases or as a means of supporting the diagnosis in cattle showing suggestive symptoms. For this purpose avian tuberculin or johnin have been used subcutaneously, intravenously or intradermally. Conclusive evidence as to the value of such tests for detecting infected animals lies, of course, in the demonstrating of Johne's bacilli in the intestinal wall or glands, but for several reasons this evidence is not always obtainable. One difficulty is the cost of bringing the animal to *post-mortem*. Other reasons are that there is no convenient animal for test inoculation, that infected animals do not always show distinct macroscopic lesions, and that even on microscopic examination of films from the intestine or glands acid-fast bacilli may not be found. In order to establish the diagnosis, therefore, it may be necessary to make histological preparations or to attempt cultural isolation of the organism. In the latter case there is, of course, some technical difficulty and especially is this so in early cases.

The objects of the work set out as briefly as possible in this paper were to study the behaviour of animals infected with Johne's disease when subjected at intervals to a number of intradermal tests with johnin, and to examine them after death for Johne's disease and tuberculosis. Nearly all the cases to be described were derived from seven herds previously reported (Minett, 1933) in which johnin testing of all the adult stock had been carried on as a routine over a period of about two years, usually at intervals of about three to four months.* In six of these herds Johne's disease was known to be causing loss and of a total of 499 adult animals present at the first or second test 16 per cent. gave clearly positive reactions and 72 per cent. did not react. In the series of 39 animals mentioned below where the *post-mortem* examination for Johne's disease was positive, 21 were tested three to five times, one was tested seven times while 17 could be tested once or twice only. It was not always possible to apply more than one or two tests, either because the animals were approaching an advanced clinical stage and, in consequence, were disposed of, or because the owners were averse to keeping cattle which were known to be

* The tests were carried out with the assistance of Messrs. R. S. Roberts, W. Orr and A. J. James.

reactors. This, however, afforded an opportunity of purchasing reactors while they were still in good or fair condition and of killing them for immediate examination. The following classes of animals are considered :—

(1) Thirty-nine, which had shown a definite reaction to johnin on at least one occasion and which were proved on *post-mortem* examination to be affected with Johne's disease.

(2) Seven, which had shown a definite reaction to johnin but which were not proved on *post-mortem* examination to be affected.

(3) Seven, which had shown a definite reaction to johnin but which for various reasons could not be examined *post-mortem*. The subsequent history, however, strongly suggested that they were affected with Johne's disease.

(4) Six animals, which had not reacted, but which were found *post-mortem* to be affected.

Of the animals in classes 1 and 2, 13 remained throughout in fair bodily condition and five were fat ; 17 others showed symptoms shortly before slaughter. In seven cases the bodily condition was noted to vary from time to time, there being periods of unthriftiness with some looseness of the bowels. Four were clinical cases from the start. Fourteen of the animals were kept under observation at the Institute for periods varying from three to six months and in two cases as long as a year, partly in order that their clinical condition might be followed, but particularly so that further tests might be made with johnin, tuberculin, or with avian tuberculin.

The johnin used was a ten-fold concentrated product, prepared by Dr. A. L. Sheather by growing a well-adapted strain of Johne's bacillus on Sauton synthetic medium containing extract of *M. phlei*. This product was selected partly as the result of a preliminary test on infected cattle and partly as being less likely to cause non-specific reactions than one prepared on a medium containing animal fluids. It was injected according to the method first suggested by Dunkin (1928), into the skin of the neck in dose of 0·2 c.c. followed 48 hours later by a similar injection at the same site. All injections and readings were made by myself. Caliper readings in millimetres were taken prior to injection and again 48 hours and 72 hours after the first injection, when notes were also made of the character of any swellings. In some of the earlier cases a single injection of 0·2 c.c. johnin was made into one of the anal folds, with or without a simultaneous injection in the skin of the neck. In the summarised notes below no details are given to indicate the intensity of the reactions as this would take much space and would appear to be unnecessary. It may be assumed, however, unless there is a statement to the contrary, that the reactions were unmistakably positive, *i.e.*, a swelling at the 72nd hour reaching at least twice the normal skin thickness at that point, with diffuse and rather soft œdema and sometimes slight

pain. Reactions described as " slight " or " indefinite " were those which fell short in some particular, *e.g.*, where the maximal thickening attained was rather less than twice that of the normal skin or where the œdema was not diffuse and lacked the easily compressible texture of a typical reaction. The anal fold test was considered positive if the injected fold was two to three times the normal thickness or more at the 48th hour. On some occasions the animals were tested at the same time by the single intradermal method with ordinary tuberculin or with avian tuberculin, concentrated products from glycerine broth cultures being used in dose of 0·1 c.c.

In all cases, save nine, examined after death, the animals were slaughtered at the neighbouring abattoir and always in the presence of a member of the Institute staff. The intestine, intestinal and other glands were immediately brought back to the laboratory and dealt with the same day.

The Examination for Tuberculosis and Johne's Disease.

The following in outline was the procedure ordinarily adopted in making the *post-mortem* examination.

Tuberculosis.—When no tuberculous lesions were visible to the naked eye, guinea-pigs were inoculated subcutaneously with material from the lymphatic glands after the surfaces of these organs had been cauterised. The glands of the intestinal tract were often treated separately, two guinea-pigs being injected with material from several of these glands. Sometimes guinea-pigs were inoculated with material from thoracic lymph glands only, at other times tissue from thoracic, pharyngeal, submaxillary and intestinal glands was pooled before injection. Inoculations were also made from tissues the tuberculous nature of which was in doubt. The animals were killed for examination two months later. When premature death took place nine days or more after inoculation, two guinea-pigs were inoculated from the local lymphatic glands and sometimes also from the spleen of the first. Premature deaths among the guinea-pigs were not a source of much trouble. Of 58 guinea-pigs inoculated from non-tuberculous tissues, eleven died prematurely and of these only two died before the ninth day.

Johne's Disease.—The examination for Johne's disease was made on lines much like those adopted by previous workers (*e.g.*, M'Fadyean, Sheather and Edwards, 1912). The intestine was separated from the mesentery, spread out on long tables, opened, and the contents removed with water from a hose. The appearance of the mucous membrane was then noted, smears to be stained by Ziehl-Neelsen method were taken from various parts of the small and large intestines and small pieces of the wall were fixed in 10 per cent. formalin. In this part of the examination special attention was paid to the ileo-cæcal valve and ileum and to any areas which had a suspicious appearance. Lengths of intestine

measuring some ten inches were then removed from the same parts and, together with some of the intestinal glands, were used for the cultural examination. For this purpose the bowel was thoroughly washed with tap water, the superficial layers of the mucosa from several areas scraped with a blunt knife and transferred to a sterile mortar. In the case of the glands the surfaces were cauterised and material from the interior removed to a second sterile mortar. From other glands films were prepared and portions of the tissue transferred to fixative. The material in each mortar was crushed into a paste, sterile sand being used in some cases, and was then mixed with about 40 c.c. sterile saline. Each suspension was then divided between two centrifuge tubes of 80 c.c. capacity and to one tube in each set was added an equal volume of 20 per cent. antiformin, the other tube in each set receiving a similar amount of 40 per cent. antiformin. The suspensions were left at room temperature for 30 minutes, being shaken at intervals, and the tubes were then filled with sterile saline, centrifuged, the supernatant replaced with fresh saline and, after shaking, again centrifuged. The deposit was used for making films and for sowing tubes of liver extract-phlei-egg medium, prepared in the manner described by Dunkin (1928) except that gentian violet was omitted. In some instances, a liquid medium was used, consisting of equal parts of liver extract and broth and containing glycerine extract of phlei and a small quantity of potato mash. Two to four tubes of medium were seeded from each deposit; sometimes, in addition, tubes of medium were seeded direct from the antiformin mixtures at intervals according to the method of A. S. Griffith (1916). The culture tubes were tightly capped and incubated at 38° C. for six to eight months, being inspected at monthly intervals. If a growth resembling that of the Johne's bacillus appeared, this was examined microscopically and if acid-fast organisms were present a subculture was made to the phlei-egg medium and to plain egg and a portion inoculated to one or two guinea-pigs subcutaneously. Acid-fast organisms of the proper morphology, which grew very slowly on the phlei-egg medium and which failed to grow on plain egg and which produced no lesions in the guinea-pigs killed two months later, were accepted as Johne's bacilli.

Reactors Proved on Post-mortem *Examination to be Affected with Johne's Disease.*

(*Note.*—In the following account cases where the disease was found to be at an early stage are placed towards the end of the series. The first johnin test was made on " day 1." Abbreviations used are :—R = reacted ; NR = did not react (in both cases, unless otherwise stated, the reference is to johnin used by the double intradermal method in the neck) ; K = killed ; T = tuberculosis ; J = Johne's disease or Johne's bacilli ; gp = guinea-pigs.

The indication as to the animal's age has reference to the date of the first test. Statements as to the presence or absence of lesions of T or J refers to naked-eye examination only. A test for tuberculosis by inoculating guinea-pigs was made only where stated.)

1.—Guernsey. Age unknown. Showing wasting and diarrhœa. R 1 (also avian tuberculin). NR 1 (tuberculin). K 4. No T lesions and 2 gp inoculated from bronchial glands were not infected. Advanced lesions of J in intestine, J seen in films of intestine and cultivated therefrom.

2.—Jersey cow. Age unknown. Scouring rather badly and thin. R 1. NR 1 (tuberculin). K 5. No T lesions. Ileo-cæcal valve very congested and smears from it showed scanty J, J also seen in sections and cultivated from the intestine.

3.—Black poll steer, about 2 years. R 1. Slight R 1 (tuberculin). Thin, badly-developed animal. K 15. No T lesions. Advanced lesions of J in intestine and J seen in films. Cultures not made.

4.—Jersey. Second calver. At times was in poor condition and showed some diarrhœa and falling-off in milk yield. R 1 (anal), 57, 87 (anal). R 140 (tuberculin). K 168. T lung and bronchial glands. No lesions of J, but J present in films. Sections of the ileo-cæcal valve showed very numerous J and typical histological changes. Cultures were not made.

5.—Guernsey cross. Aged cow. In fair condition at first. R 1 and 101. Slight R 21 (tuberculin). K 166 and prior to this had scoured and become emaciated. Found to be pregnant. Lesions of T in lungs and glands and on pleura. Advanced lesions of J in intestine and J seen in large numbers in films and sections from intestine. Cultures not made.

6.—Shorthorn heifer, rising 2 years. NR 1, R 101 and 214. NR 214 (tuberculin). K 250 at time when starting to waste. No lesions of T seen and 2 gp injected from the bronchial, pharyngeal and submaxillary glands, and 2 gp injected from the intestinal glands did not become infected. Lesions of J in the intestine, small numbers of J seen in films and in large numbers in sections of the small intestine. Cultures not made.

7.—Shorthorn heifer, about 18 months old. NR 1, indefinite R 101, R 214, NR 311. R (tuberculin) 21 and 214. Wasted after day 311 and K 399. The intestine and glands alone received for examination. Extensive lesions of J in intestine, J seen in films and isolated in cultures from the intestine.

(*Note.*—" Indefinite R 101 " = increase of skin thickness from 9 to maximum of 16 mm., but the swelling was firm and circumscribed.)

8.—Cross-bred cow. NR 1, 97 and 206, R 306. NR (tuberculin) 1 and 206. Calved day 295 and then began to scour and waste. K 316. No T lesions and 2 gp injected from bronchial and intestinal glands did not become infected. Extensive lesions of J in intestine, J seen in antiformin preparations, in sections and isolated in culture.

9.—Cross-bred Shorthorn cow. Age unknown. Bad clinical case. R 1 (also tuberculin). Slight R 1 (avian tuberculin). K 8. No T lesions. Lesions of J in intestine, numerous J seen in films from intestine and cultivated therefrom.

10.—Shorthorn cross. Yearling heifer. R 1, 97 and 206, NR 311. R 206 (tuberculin). Started to waste just prior to day 353 when K. T lesions present in submaxillary gland only and 2 gp inoculated from it became infected. 2 gp inoculated from intestinal glands did not become infected. Advanced lesions of J were present in the intestine, J were seen in films and in enormous numbers in sections of the intestine and were isolated in culture.

11.—Guernsey heifer, rising two years. NR 1, slight R 101, R 214 NR 311, R 401. R (tuberculin) 21 and 214. In poor condition and scouring after the test of day 311. K 409. T lesions present in bronchial glands; gp inoculated from the intestinal glands did not become infected. Lesions of J in intestine; histologically there were extensive alterations and very large numbers of J; cultures were positive.

12.—Shorthorn bull. 3½ years old. R 1 (anal). In good condition for 186 days when active signs of J developed. K 195. No T lesions. Lesions of J in intestine and J in films, sections and cultures of intestine.

13.—Guernsey heifer, rising 2 years. In fair condition at first. R 1. Slight R 22 (tuberculin). Reported to be very thin and scouring on day 92. K 102. No T lesions. Lesions of J in intestine, J in films of intestine and isolated in culture.

14.—Shorthorn. Second calver. In fair condition. R 1, NR 92 (also anal). R 1 (tuberculin), NR 8 (subcutaneous tuberculin). Became very emaciated by day 92. K 99. No T lesions. Lesions of J in intestine and J in films of intestine and isolated in culture.

15.—Shorthorn heifer. About 2 years old. R 1, slight R 97, when reported to be scouring. NR 21 (tuberculin). Carcase in moderate condition when K on day 158. No T lesions. Lesions of J in intestine, J seen in films from intestine and isolated in culture.

16.—Shorthorn. Yearling heifer. NR 1, R 97 and 204. NR 21 (tuberculin), R 204 (tuberculin). K 220. No T lesions. Lesions of J in intestine, J seen in films from intestine and isolated in culture.

17.—Jersey heifer. From alleged T-free herd. R 1 and 97. NR 1 (tuberculin). K 155. No T lesions and 2 gp inoculated from pharyngeal and intestinal glands failed to become infected. Unfortunately, material from the bronchial glands was not inoculated. Lesions of J in intestine and J seen in antiformin preparations and isolated in culture.

18.—Shorthorn heifer. Nearly 2 years old. Scouring at times, latterly rather badly, but did not become emaciated. Slight R 1, R 92 (also anal), and 175, NR 267. R (tuberculin) 1 and 175. K 273. One mediastinal gland contained calcerous areas, which could not be demonstrated by gp inoculation to contain tubercle bacilli. 2 gp inoculated from the bronchial, pharyngeal, submaxillary and intestinal glands did not become infected. Lesions of J in intestine, J seen in films of intestine and isolated from intestine.

(*Note.*—" Slight R 1 " = increase of skin thickness from 6 to maximum of 12 mm., but the swelling was quite firm and well-defined.)

19.—Shorthorn. 3 years old. R 1, 113 and 210. R (tuberculin) 113 and 210. K 246. No T lesions. 2 gp were inoculated from bronchial, pharyngeal and submaxillary glands, 2 from the submaxillary gland and 1 from the mesenteric glands. None of these animals became infected. Slight congestion of the ileo-cæcal valve, J seen in antiformin preparations and in sections of intestine and cultivated from both intestine and mesenteric glands.

20.—Shorthorn. Yearling heifer. NR 1, 101, 311, 401, 486 and 575, R 214. NR 575 (avian tuberculin). NR (tuberculin) 214 and 575. Wasted and showing diarrhœa after day 486. K 579. No T lesions and 2 gp inoculated from bronchial and mediastinal glands did not become infected. Typical lesions of J present in intestine, J seen in films from intestine and gland and were cultivated from these sites.

(*Note.*—" R 214 " = increase in thickness of skin from 6 to 17 mm.,

swelling diffuse. At the other tests a maximal thickening of a shade over twice the original was reached on two occasions only, but on one of these the concentrated medium was used as a control and gave practically the same reading.)

21.—Friesian. Third calver. From alleged T-free herd. R 1 and 11. K 44. Scouring badly a few days before slaughter. No T lesions and 3 gp inoculated from bronchial, pharyngeal, submaxillary and mesenteric glands did not become infected. Lesions of J in intestine, J seen in films from intestine and cultivated therefrom.

22.—Friesian. Second calver. From alleged T-free herd. R 1. K 44. Scouring before slaughter. No T lesions and 2 gp inoculated from bronchial and mediastinal glands did not become infected. Lesions of J in intestine, J seen in films from intestine and cultivated therefrom.

23.—Shorthorn. Third calf, day 163. R 1, 85, 154, 225 and 246. R (tuberculin) 1, 85 and 225. Remained in fair condition. K 295. T lesions present in bronchial glands; 2 gp inoculated from the intestinal glands did not become infected. Lesions of J present in intestine, J not seen in antiformin preparations of intestine and intestinal gland, but were isolated from both situations in culture.

24.—Shorthorn cow. NR 1, R 85 (anal). NR (tuberculin) 1 and 85. Had wasted before test on day 85. K 92. T lesion in one mesenteric gland, inoculation of which produced T in gp. Lesions of J not definite; J seen in antiformin preparations and in small numbers in sections from the small and large intestine; J cultivated from an intestinal gland.

25.—Shorthorn cow. Aged. Had shown intermittent but not copious scouring. R 1 and 57. R (tuberculin) 1 and 57. Calved day 109. K 147. Lesions of T in lungs and bronchial glands and in one or two of the mesenteric glands. Ileo-cæcal valve showed some patchy congestion; J seen in antiformin preparations of the intestine and in very small numbers in sections of the small intestine; J isolated in culture.

26.—Shorthorn cow. R 1 (also anal), 101 (also anal), 214. R (tuberculin) 1 and 214. K 250, when starting to lose condition. Animal pregnant. No T lesions seen and 2 gp injected from bronchial, pharyngeal and submaxillary glands and 2 gp from intestinal glands did not show infection. Slight lesions of J in intestine, a few J seen in films and sections of intestine and J were isolated from intestine.

27.—Red poll. From alleged T-free herd. R 1 (anal), NR 91 (anal), R 202 and 343. In poor condition and very loose for some time prior to slaughter on day 351. No T lesions seen and 2 gp injected with material from bronchial glands showed no T lesions when killed. Lesions of J in intestine, J seen in antiformin preparations and in very small numbers in sections of the intestine and isolated from the intestine.

28.—Cross-bred heifer. R 1, 101, 214, 311 and 401. R (tuberculin) 21 and 214. In fat condition when K on day 430. No T lesions and 2 gp inoculated from bronchial, pharyngeal and intestinal glands did not become infected. Ileo-cæcal valve was congested; J were seen in antiformin preparations, were easily found in sections of the intestine, but were not cultivated therefrom.

29.—Shorthorn cow. Aborted first calf and lost much condition. In good condition on day 1. Second calf, day 69, after which wasted somewhat. R 1, 57, 154 and 225. NR (tuberculin) 1, 154 and 225. K 233. No T lesions seen and 2 gp inoculated from the bronchial, pharyngeal and intestinal glands did not become infected. No lesions of J and no J seen in antiformin preparations of intestine. J cultivated with great difficulty from intestine.

30.—Shorthorn. Second calf, day 45. Poor condition after first calf, but improved and remained in fair condition after second calf, though scouring at times. R 1 (also anal), and 57. NR 154 and 225. R (tuberculin) 1 and 154. NR (tuberculin) 225. K 233. No T lesions and 2 gp injected from the bronchial, pharyngeal and intestinal glands did not become infected. Very slight congestion of ileo-cæcal valve, but no J seen in antiformin preparations of intestine, J isolated in culture.

31.—Shorthorn. From alleged T-free herd. Second calf day 283. Rather unthrifty condition throughout. NR 1 (anal), R (anal) 123 and 227, slight R 346. NR (tuberculin) 225. K 501. No T lesions and 2 gp injected from the bronchial glands did not become infected. Ileo-cæcal valve deeply congested, but no J seen in films. Sections of the intestine showed very small numbers of acid-fast bacilli like J and slight histological alterations. J were not isolated in culture.

(*Note.*—" Slight R 346 " = increase in skin thickness from 6 to maximum of 11 mm., swelling soft and diffuse.)

32.—Red poll. From alleged T-free herd. First calf day 165. In fair condition prior to calving, though scouring at times ; after calving wasted considerably, but later much improved. R 1 (anal) ; R 126, 217, 314 and 406. NR (tuberculin) 55, 126 and 314. K 412. No T lesions present and 2 gp injected from bronchial, pharyngeal, submaxillary and intestinal glands failed to become infected. Slight lesions of J present, a few J seen in films of intestine and J isolated in culture.

33.—Cross-bred cow. Age unknown. Fair condition. Calved day 119. R 1, 175 and 280. NR (tuberculin) 1, 175 and 280. K 310. No T lesions seen and 2 gp injected from bronchial, mediastinal, pharyngeal and submaxillary glands and 2 gp injected from intestinal glands did not become infected. Slight congestion of the ileo-cæcal valve. No J in antiformin preparations of the intestine, but J isolated in culture.

34.—Shorthorn heifer. NR 1, R 101, 214 and 311. NR 401. R 214 (tuberculin). In fat condition when K on day 430. T lesions present in lungs, bronchial and intestinal glands. No J lesions seen in intestine and no J seen in antiformin preparations of intestine, but J were isolated from the intestine in culture.

35.—Jersey. Third calver. From alleged T-free herd. Slight R 1. R 1 (avian tuberculin). K 117, when very wasted. Intestine alone examined, no lesions of J present but small numbers of J seen in films from ileum and cultivated therefrom.

(*Note.*—" Slight R 1 " = increase of skin thickness from 6 to 10 mm. with soft œdema.)

36.—Red poll. First calver. From alleged T-free herd. R 1. K 126. Intestine alone examined, no lesions of J present, but J seen in films from intestine and cultivated therefrom.

37.—Shorthorn. First calver. From alleged T-free herd. Good condition throughout. R 1 (anal), 18 (anal), 99, 201. NR 152 (tuberculin). K 208. No T lesions. No lesions of J and no J in films. 28 sections from 10 sites in the small and 5 sites in the large intestine were searched but no J were seen and there were no characteristic histological changes. J, however, were isolated in culture.

38. Shorthorn cow. Remained in good condition. R 1, 92 (also anal), 190 and 267. The reaction on day 1 was slight. Indefinite R (tuberculin) 1, NR (tuberculin) 8 (subcutaneous), 190 and 267. K 310. No T lesions and 2 gp injected from bronchial, mediastinal, pharyngeal and submaxillary

glands and 2 gp injected from the intestinal glands did not become infected. Slight congestion of the ileo-cæcal valve and early J lesions present in ileum. No J seen in antiformin preparations of intestine or in sections but J isolated in culture.

39.—Ayrshire cow. 4½ years. From alleged T-free herd. R 1 and 92. R 92 (tuberculin). K 15. No T lesions seen in 2 gp inoculated from bronchial, pharyngeal and submaxillary glands and 2 from intestinal glands did not become infected. No lesions of J seen, though the mesenteric glands were rather watery. No J seen in films or sections from small or large intestine or mesenteric glands, but J colonies appeared in small numbers on media sown with material from small and large intestine.

Reactors in which the Existence of Johne's Disease Post-mortem *was Not Proved.*

Seven cases fall under this heading, though with 4 of them the diagnosis *post-mortem* was doubtful. With two exceptions, all were adults. All seven animals had definitely reacted to johnin and were purchased from herds in which Johne's disease was known to exist. Most of the cases were dealt with quite early in the work and a sufficiently extensive examination was not made.

40.—Had not reacted to tuberculin and no evidence of T *post-mortem.* In a smear of the small intestine after much search two or three clumps of acid-fast bacilli rather like J were seen. Antiformin deposits prepared from the ileo-cæcal valve, small and large intestine were injected into gp but did not set up T. No definite changes and no J were found in sections from the small or large intestine. Cultures were not attempted.

41.—Had shown intermittent diarrhœa. From an alleged T-free herd and no evidence of T *post-mortem.* In a smear from the ileo-cæcal valve after much search two small acid-fast rods were found. Cultural examination for J was fairly complete, but the result was negative.

42.—Very thin at time of death and had not been thriving. T lesions present. The ileo-cæcal valve was slightly congested. Films from the intestinal wall were negative but those from antiformin deposits showed a few clumps of acid-fast bacilli, many of the organisms resembling J. No material was taken for histological examination. Cultural examination was extensive, but the result was negative.

43.—A bull. Did not react to tuberculin and no T lesions seen *post-mortem.* Ileo-cæcal valve deeply congested. Cultural examination was extensive and many fruitless attempts were made by periodic subculture to demonstrate J. After 8 months' incubation a tube of the liver extract-broth medium showed slight scaly surface growth in which acid-fast bacilli were present but no subcultures could be obtained.

44.—No evidence of T. Antiformin preparations showed no acid-fast bacilli microscopically, sections from three different sites in the ileum gave no indication of J. The cultural examination was not extensive.

45.—A yearling calf, badly affected with T. Unfortunately, most of the cultures from intestine and gland were contaminated.

46.—A yearling calf, badly affected with T. Cultural examination of the intestine and glands for J was extensive, but the result was negative. This animal was from a group of 6 calves, 2 of which were proved later to be infected with avian tubercle bacilli. The johnin reaction in this case may have been due to infection with either avian or bovine tubercle bacilli.

Reactors in which the Previous or Subsequent History Suggested Johne's Disease.

During this work there have been a small number of animals which for various reasons could not be brought to *post-mortem* examination, but where the history was suggestive of Johne's disease. Such cases go to substantiate the value of johnin, but do not, of course, carry as much weight as those which could be thoroughly examined after death.

47.—Shorthorn. Second calver. R 1, 84, 195, 293 and 426. Calved a few weeks after day 426. Reported by the owner to have died a few days after calving, greatly wasted, due probably to Johne's disease.

48.—Shorthorn cow. Belonged to same herd as No. 47 and mother believed to have died of J. R 1, 98, 293, 391 and 524; NR 182. NR 524 (tuberculin). Reported to have lost flesh rapidly by day 674 and the butcher found it to be " one of the worst cases ever opened."

49.—Shorthorn. First calver. R 1, when thin and scouring. Second calf day 61. Sold before day 90 " with every appearance of Johne's disease."

50.—Shorthorn. First calver. Reported by owner to have scoured badly for a time.

51.—Shorthorn. Second calver. Reported by owner to scour at times.

52.—Shorthorn. Third calver. Reported by owner to have scoured badly for a time.

Nos. 50, 51 and 52 belonged to the same herd.

53.—Friesian. Third calver. R December. Reported to have been scouring during the previous summer.

Non-Reactors Found to be Affected with Johne's Disease.

It is to be noted that three of these were tested once only and that one of the remainder was an advanced clinical case from the start.

54.—Welsh heifer. Good condition. NR 1 (anal). Showed symptoms shortly after. K 48. No T lesions. Advanced lesions of J in intestine. J seen in smears and sections of intestine and isolated in culture.

55.—Welsh heifer. Very poor condition. NR 1 (also tuberculin). K 15. No T lesions. Advanced lesions of J in intestine and numerous J in films therefrom.

56.—Shorthorn cow. Fair condition. NR 1 (anal). Owner suspected J shortly before slaughter. K 295. No T lesions. Slight lesions of J in intestine; examination of sections showed numerous giant cells and small numbers of scattered J; J isolated in culture.

57.—Ayrshire heifer, in first pregnancy. Very poor and scouring. NR (also anal), 1, 111 and 132. NR 132 (tuberculin). K 141. No T lesions. No definite J lesions and no J found in films of intestine. In sections of the intestine there were numerous giant cells, but only three small acid-fast bacilli like J could be found after much search. J isolated in culture.

58.—Shorthorn cow. Fair condition. NR 1 (also anal) and 62. Slight R 22 (tuberculin), NR 43 (tuberculin subcutaneous). Wasted rapidly after day 1. K 66. T lesions found in one submaxillary gland only and gp inoculated from it became tuberculous. Ileo-cæcal valve congested and slight lesions of J in ileum. J seen in films and in very large numbers in sections of the small and large intestine and were isolated in culture.

59.—Shorthorn heifer. Fair condition. NR 1 and 101. Thin at time of second test and became very emaciated by day 199 when K. No T lesions. Advanced lesions of J in intestine, J seen in films and in enormous numbers in sections of intestine and cultivated therefrom.

Discussion.

Reports in the literature dealing with the value of johnin, prepared in various ways and injected in most cases by the subcutaneous or intravenous route are given by Twort and Ingram (1913), M'Fadyean, Sheather and Edwards (1916), Beach and Hastings (1922) and others. The diagnosis *post-mortem* was based on the presence of lesions visible to the naked eye and on the finding of acid-fast bacilli in smears of the intestinal mucous membrane. As to the use of johnin intradermally, Broerman and Fogle (1932) reported the results of tests by the single injection method. Definitely positive reactions were obtained in 27 animals and of these 19 were brought to *post-mortem*. A positive diagnosis by microscopic examination of films from the intestine was made in twelve of the 19, but slight or marked naked-eye lesions were present in three of the others. Of the eight animals not brought to *post-mortem*, one was a clinical case. Dunkin (1928) refers to 17 reactors, of which the fate was known and which were detected in field tests by the double intradermal method. Of these eight were clinical cases and the remainder were found to be affected with Johne's disease on *post-mortem*. Houthuis, whose results of johnin testing were reported in 1932, has made it clear to me by private communication that he examined *post-mortem* 42 cattle of which 38 were found to be affected with Johne's disease. The diagnosis was based on an exhaustive histological examination of the intestine and glands, since macroscopic evidence of disease was absent in several cases. Johnin testing was carried out by the double intradermal method. Forty-one of the animals were tested with johnin prepared by Sheather on the Sauton synthetic medium and of these 27 reacted; eight of the non-reactors, however, were advanced clinical cases and the other four non-reactors were positive on *post-mortem* examination. Twenty-eight of the 42 were tested with johnin prepared by Dunkin according to the method described by him in 1928; of these 25 reacted, two of the non-reactors were advanced clinical cases and the remaining one was found to be affected on *post-mortem* examination. Of the 27 reactors to Sheather johnin 25 were found to be affected with Johne's disease and two with tuberculosis only. Of the 25 reactors to Dunkin johnin 24 were found to be affected with Johne's disease and one with tuberculosis only.

The results obtained with the series of 59 animals now recorded are summarised in Table I.

Table I.

Post-mortem Examination.	*Johnin.* +	*Johnin.* —
+	39	6
+	7	
No p.m. History suggestive of Johne's disease ...	7	
	53	6

From this it may be seen that of 46 reactors which were examined *post-mortem* 39 were definitely proved to be infected with Johne's disease. The remaining seven animals were dealt with at an early stage of the work and the *post-mortem* was incomplete; nevertheless, in four of them acid-fast bacilli which may have been those of Johne's bacilli were met with. In three of the 39 cases, *viz.*, Nos. 37, 38 and 39, the disease was at a very early stage and a definite diagnosis was effected by cultural methods only. In two cases (Nos. 28 and 31) cultural methods failed and a diagnosis was established by finding acid-fast bacilli in films or sections of the intestine. Altogether, of the 53 animals which reacted, 39 were definitely infected and there is evidence to indicate that eleven of the remaining 14 were also infected. Of the six animals which did not react, three were tested once only and four of the six were at an advanced clinical stage. Out of the whole series of animals two were Ayrshires, a breed not commonly reported as infected; these belonged to a herd in England which was being recruited with this breed.

In those infected animals which were subjected to several retests it was found that strong reactions were not produced every time. Occasionally, on retest the reaction was slight or indefinite, or even negative. In this connection special attention should be directed to Case 20. There may, of course, be differences of opinion as to the interpretation which should be placed on slight swellings, but this does not alter the conclusion that much variation may occur in the degree of local response. The differences may be due either to variations in the potency of the johnin used or to variations in the allergic state of the animal. The former explanation seems unlikely for several reasons. Firstly, the reagent was always made in the same way and by the same person, and variation in its properties would be less likely with a synthetic medium than with one containing albuminous fluids. The strain of Johne's bacillus used for seeding the medium gave rich growths on it and the contents of four flasks were mixed before the cultures were concentrated. Secondly, some quite definite positive reactions were obtained with every batch of the reagent used. With regard to the second explanation, it is agreed that the allergic condition created by the Johne's bacillus is relatively low, *e.g.*, as compared with that induced by the tubercle bacillus; moreover, it would not

be expected that an infected animal, even though it remained in fair condition, would maintain the same level of allergy at all times. The fact that cattle affected with Johne's disease may react well at one test and indefinitely at a subsequent one has been noted by others, though no extensive series of repeat tests has been reported. This is one of the reasons why repeated testing would be required in any serious attempt to eradicate the disease.

There was nothing to show from the *post-mortem* examinations that the reactions to johnin were connected with tuberculous infection. Tuberculous lesions were found in eight of the 39 animals, were not found in 26, and in five cases a complete *post-mortem* was not possible. It may be noted that nine of the 26 animals referred to did show what was interpreted as a positive reaction to tuberculin, but in four of the nine animals control tests on guinea-pigs were not made. As it happened, however, in the present series tuberculosis was not set up in any guinea-pig inoculated from apparently healthy glands.

Summary.

An account is given of 59 cattle of which 52 were examined *post-mortem* for Johne's disease and many for tuberculosis. Fifty-three of the 59 animals had reacted to the double intradermal test with johnin, many of them on more than one occasion. Thirty-nine of the 53 were conclusively proved to be infected with Johne's disease, while there was presumptive evidence of this infection, either from *post-mortem* examination or from the history of the case, in eleven of the remaining 14. Six of the 59 animals did not react to johnin, but three of them were tested once only and four of the six were at an advanced clinical stage.

Repeat tests with johnin on a number of the animals showed much variation in the degree of local response, a result which is attributed to variations in the allergic state.

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THE AUTHORSHIP OF CARLO RUINI'S "ANATOMIA DEL CAVALLO."

By H. P. Bayon.
Henry George Plimmer Fellow in Pathology.

Several reasons justify the desire of ascertaining the authorship of the drawings and text of the book on the anatomy of the horse first printed in Bologna in 1598 under the name of Carlo Ruini. This work was not only the first, but remained for a long time the best and most complete, treatise on equine anatomy and medicine; accordingly it was extensively copied and indeed became the basis of all subsequent advances in knowledge of the subject. Then the text contains views on the pulmonal and general circulation of the blood, which at the time were certainly revolutionary, even if in the main correct. Moreover the artistic excellence of some of these anatomical tables was not approached, even when reproduced; not till the appearance of the remarkable engravings of George Stubbs (1724-1806) published in 1766; whose splendid illustrations of the muscles of the horse, representing dissections made by the artist, were not completed by representations of the inner organs. Modern works on equine anatomy have been able to take advantage of methods of preservation and aids to dissection, together with photography and microscopy and are therefore more accurate and detailed. Nevertheless, for innate fire, artistry and animation the woodcuts of Ruini's work can still be considered supreme.

The illustrations of this book being obviously the work of a supreme craftsman, the desire to learn something about the life of the author was natural. Very little has been found out about Carlo Ruini, except that he was a lawyer and a Senator of Bologna; the date of his birth is unknown, that of his death uncertain, but can be assumed to be about 1598. The first edition of the treatise appeared in two volumes, the first of which was entitled " Anotomia del cavallo," and the second " Dell 'infirmità del cavallo " (Fig. 1). It will be noticed that the spelling of the word anatomy on the title page is erroneous, though correct elsewhere in the text. The printers are: Heredi di Giov. Rossi. The book is dedicated to Cardinal Pietro Aldrobandini, a nephew of Pope Clement VIII and Superintendent of the Pontifical Army. The preface of the first volume is dated 7 January, 1598, and is signed: Carlo Ruini. In this preface he states that it was an ancient custom of the Romans to consecrate after long service their horses to the gods and eternal repose; so it is traditionally reported that Cæsar, after passing the Rubicon, dedicated his well-deserving steed to Venus genitrix, from whom he pretended

to descend. In imitation of this example, Ruini continues, I have placed to rest my horse which has carried me about for many years. The writer then proceeds in a flowery panegyric of Cardinal Aldrobandini. In subsequent pages follows a preamble or " proemio," which opens with a praise of the horse and an appreciation of its usefulness in business, war and pleasure. Here Ruini says that he has ridden since childhood; he further states that he has studied the anatomy of the horse for the purpose of dealing with the diseases in a manner which would satisfy him and which, up to the present, had not been done. The anatomical portion of the work consists of 295 pages divided in five chapters. It may be mentioned that Table V bears on a tablet the clear date: MDXC.

The second book, which describes the diseases of the horse, also contains some prefatory notes, but these are of a general nature and not signed; the date is also 1598, but it may be that in the meanwhile Ruini had died. It consists of six chapters or " libri " and 386 pages.

This first edition is in the British Museum, where the two books are in one volume in a modern binding and wrong sequence, because the anatomy is obviously the first. This first edition came to notice after I had remarked in a previous paper in this journal that in my opinion the Venetian edition of 1599 (of which several specimens exist) was the first; being misled by the statement on the title page: " Opera nuova " and in the preface by the printer, Gasparo Bindoni, who says that he had obtained the text from the heirs of Ruini for the purpose of printing it. One may therefore presume that Ruini had died some time before. This mistake is shared with others, for example Sir Frederick Smith (1919) and Dr. Schmutzer (1913); the latter author, in a paper entitled " Bemerkungen zur Pferdeanatomie des Lionardo da Vinci," writes that the " Auskunftsbureau der deutschen Bibliotheken " had informed him that the Bologna 1598 edition was nowhere to be found. Similar views are expressed in several papers dealing with the subject and for this reason a photograph of the title page of the second volume of the Bologna edition is here reproduced, from the copy in the British Museum. (Fig. 1.).

The excellent illustrations will receive notice later on, but in the meanwhile the text claims attention, for by frequent comparisons and references it reveals a good knowledge of human anatomy; for example, in relation to the heart, eye and brain. As to the latter, the size of the " buttocks " of the human brain (in modern terminology, the hemispheres) is compared with those of the horse's brain; a rather long disquisition follows explaining that man having so much more imagination and phantasy, he requires larger organs to cool them down.

Then one may refer to the passage which has made some authors consider Ruini as a precursor of Harvey in the discovery

of the circulation of the blood. The few lines in question are found on pages 108-109 and may be thus translated: "The office of the right ventricles is to dispose of the blood which obtains from them the spirits of life and to nourish the lungs; of the left to receive this blood already prepared and convert one part in spirits, which give life and send the remainder with the spirits through the arteries to all parts of the body." (Fig. 2.) It will be easily seen that there is little which can bear a comparison with the complete work, thoroughly reasoned out and skilfully supported by dissections of different animals and experiments on the living which stand to the credit of William Harvey (1578-1657.) These views of Ruini may well have been obtained from the work of Matteo Realdo Colombo (1516-1559) "De re anatomica" or

DELL'INFIRMITA'
DEL CAVALLO
DI CARLO RVINI
SENATORE BOLOGNESE.
ALL'ILLVSTRISS.^MO ET REVERENDISS.^MO
MONSIGNORE CARDINALE
PIETRO ALDROBANDINI.
Nepote del Santiss.^mo Clemente Ottavo Sommo
Pontefice, &c. dedicato, & consecrato alla Eccellenza sua
hoprirentemente tiene le.
IN BOLOGNA.
Presso gli Heredi d. Gio. Rossi. MDXCVI.

Fig. 1.

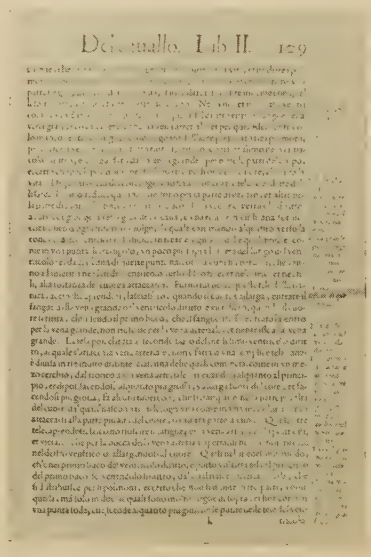
Del cavallo. Lib. II. 129

Fig. 2.

that of Andrea Cesalpino (1524-1603) entitled "Questiones medicae" which appeared in 1593. The paragraph quoted (see Fig. 2) induced the veterinarian and historian, Giovanni Battista Ercolani to consider Carlo Ruini as a forerunner to Harvey in relation to the discovery of the circulation of the blood; this view was set forth in a book printed in 1873 and a tablet was placed on the walls of the University in Bologna recording Ruini's achievement.

A few words may be said about the Venetian edition; this is entitled "*Anatomia del cavallo, infermità ed i suoi rimedi,*" here the two books appeared together under one title page. Printer: Gasparo Bindoni il giovane, Venice 1599. The preface is written and signed by Bindoni, who mentions that Carlo Ruini

was a lover and breeder of good horses and that the text and illustrations had been found among the Senator's papers and handed to him for publication by the heirs of Ruini, amongst which the son Ottavio is mentioned, who had served in France under Henri IV, to whose son the book is dedicated. Print, paper and illustrations are an improvement on the previous edition.

That the work of Ruini influenced the development of equine anatomical knowledge can be surmised from the repeated editions which appeared in 1602, 1607, 1618. The text was translated into German by the physician Peter Uffenbach in 1603 and a French translation dealing with the diseases alone appeared under the name of Horace de Francini. This translation deserves some notice from several points of view. It is entitled " Hippiatrique du Sieur Horace de Francini, escuyer ordinaire du Roy, et capitaine des garennes de Bourgogne. Paris, chez Marc Orry, MDCVII." The work is dedicated to " Messire Rogier de Bellegarde, Grand Ecuyer de France."

It should be noted that the alleged author of the book places a " de " before his name, which presumes a right to nobility. The dedication is, however, signed: Horatio D. Francino, a somewhat different version. The dedication says: " Ayant à peine attaint l'age de douze ans, le Seigneur Charlot Ruyna gentilhomme boulonnais mon Oncle, me logeant chez soy, me donna le premier ply & m'enseigna les Rudimens de sa profession; que je peux dire avoir esté l'un des meuilleurs & scavants de son siècle; ie fus chez luy l'espace de douze ans etc." Francino therefore states that he was a nephew of Carlo Ruini and continues to say that wishing to go to France he joined another relative in Lyons; where having advanced in knowledge, he wrote the book on the diseases of the horse and sent the script to Ruini for his criticism, after whose death the manuscript had been published by his heirs. He proposes to deal with the anatomical portion later on. This account explains the incorrect statements which have found place in text-books and encyclopædias; that Ruini was a veterinarian (" he taught me the rudiments of his profession ") and that the book was published after his death. One may query Francino's claim that he wrote the part relating to the diseases; his French is that of a man unused to writing, the Italian of Ruini is remarkably clear and the literary style that of an educated man. Still one cannot understand why Francino should have gone to the trouble of inventing a relationship to Ruini, when he could have easily purloined the text without further ado, unless the Venetian edition was already well known in France, which it well might be. Francino indeed claimed having written for his uncle the part dealing with diseases. It is difficult to decide whether this was the case, but it can be said that the book on the diseases and treatment does not reach the level of lasting excellence shown by the anatomical

portion. Still, it must be understood that at the time veterinary medicine was considered a branch of agriculture and that in relation to the horse, attention was concentrated on horsemanship, a great deal of which seemed to consist in the invention of new models of screw-bits and cruel methods of training, whilst the remainder was strongly tinged with quackery and superstition. If this is taken into consideration, the work of Ruini can be rightly considered enlightened, for it presents what may be called common-sense views. In the text he shows a good practical knowledge of equine ailments and reveals himself as a humane horse master; thereby differing from contemporary farriers, like Filippo Scacco (1591), whose crude illustrations are reproduced in Sir Frederick Smith's (1919) book.

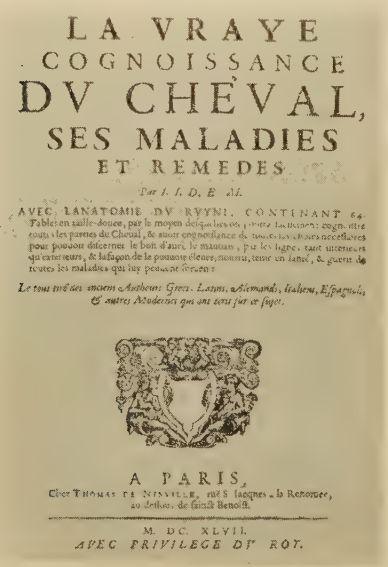
LA VRAYE
COGNOISSANCE
DV CHEVAL,
SES MALADIES
ET REMEDES
Par I. I. D. E. M.
AVEC L'ANATOMIE DV RVYNI. CONTENANT 64.
Tables en taille-douce, par le moyen desquelles on pourra facilement cognoistre
toutes les parties du Cheval, & aussi cognoistance de toutes celles dont a besoin
pour pouuoir discerner le bon d'auec le mauuais, par les signes tant internes, et
qu'externes, & la façon de le pouuoir penser, nourrir, tenir en santé, & guerir de
toutes les maladies qui luy peuuent suruenir.
Le tout tiré des anciens Autheurs Grecs, Latins, Allemands, Italiens, Espagnols,
& autres Modernes qui ont escrit sur ce sujet.
A PARIS,
Chez THOMAS DE NINVILLE, rüe S. Iacques, à la Rencontre,
au dessus de sainct Benoist.
M. DC. XLVII.
AVEC PRIVILEGE DV ROY.

Fig. 3.

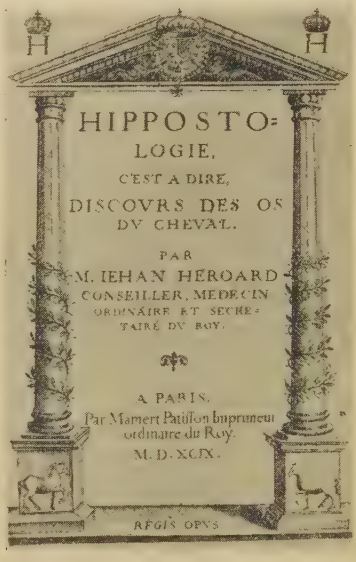
HIPPOSTO=
LOGIE,
C'EST A DIRE,
DISCOVRS DES OS
DV CHEVAL.
PAR
M. IEHAN HEROARD
CONSEILLER, MEDECIN
ORDINAIRE ET SECRE=
TAIRE DV ROY.
A PARIS,
Par Mamert Patisson Imprimeur
ordinaire du Roy.
M. D. XCIX.
REGIS OPVS

Fig. 4.

The anatomical portion was also reproduced in a French work called " La vraye cognoissance du cheval, ses maladies et remedes avec l'anatomie du Ruyni." Paris: chez Thomas de Ninville MDCXLVII. The author of this hides his name under the initials " I. I. D. E. M." which correspond to: Iean Iourdain Docteur en Médècine. (Fig. 3.) The book is remarkable from this point of view, that the preface contains the statement that the plates, which are reversed transfers from the " Anatomia del cavallo " are " une tres-ample et très-exacte Anatomie designée apres nature, par Titian tres fameux peintre de l'antiquité," etc. Titian died in 1576 and it is remarkable that Iourdain should have thought of him as the author of the drawings. This will be referred to further on, but in the meanwhile one can see how

a physician, who must have had some knowledge of clinical medicine, would approach the subject of the diseases of the horse; the approach consists in the literary method of compilation, that is by quoting one after the other various authors, like Vegetius, Apsyrtus, and so on.

It may be mentioned that the Court Physician, Jean Héroard (1561-?) published his " Hippostologie c'est a dire, discours des os du cheval " at Paris in 1599. This is a learned, well-written work, carefully illustrated and beautifully printed, dealing with the osteology of the horse. The description of the bones is good, though not better than that found in the text of Ruini and it is difficult to decide whether both authors did not borrow from the same source. The title-page is reproduced in Fig. 4. Moreover an illustration of the whole skeleton of the horse from this book is reproduced under Fig. 5; there is a definite resemblance to the corresponding plate in Ruini, though less accuracy in detail. Obviously there is not much scope for artistry in delineating dry bones and a certain similarity in all skeletons is unavoidable.

We now come to the work of Andrew Snape (1664-?), farrier to Charles II, who wrote the text of " The Anatomy of an Horse," London, 1683. It is a blot on Snape's good name that he copied (or had copied) the illustrations of Carlo Ruini and reversing them, introduced several inaccuracies in the process. However admirable his copper plates may be when compared with the woodcuts of Ruini, Snape could not truthfully state that they were original drawings.

The examples mentioned show that the work of Ruini did influence anatomical and veterinary practice in several countries for some considerable time after his death; that his contribution to the problem of the circulation of the blood was small even if important; that the excellency of his anatomical plates justify the suspicion that they must have been the work of a consummate artist working in conjunction with a skilled anatomist. The text we may admit as Ruini's possibly with a little guidance from a medically trained friend; but even that was not necessary; he may have read medical books. It has been seen that the name of Titian has been mentioned in relation to the drawings of Ruini's Anatomy: it is difficult to say on what grounds except that the supreme artistry of the illustrations was recognised.

The suggestion that the anatomical illustrations in Ruini's book are based on drawings by Leonardo da Vinci was originally made by Jackschat (1902) who considered that Ruini's work was a plagiary of the studies on the anatomy of the horse which Leonardo prepared for the Sforza monument; these studies, it is well known, lasted ten years (1483-1493) and resulted in a plaster cast, which was eventually destroyed by French soldiers which had invaded Milan in 1499. That Leonardo da Vinci has left records of many years of observations on the external anatomy of

horses is known and it would be indeed remarkable in view of the feverish activity and scientific curiosity which animated Leonardo to find that he did not go further than the surface. Jackschat, moreover, considered that Andreas Vesalius (1514-1564), for the purpose of completing his " De fabrica humani corporis," had purloined Leonardo's drawings of human anatomy, of which several books of notes and sketches are still extant. Roth (1907) opposed these views with what we may consider excessive zeal and criticised several erroneous observations in Leonardo's note-books.

Regarding the anatomy of the horse, Schmutzer (1913) pointed out that only superficial drawings of the horse were known of

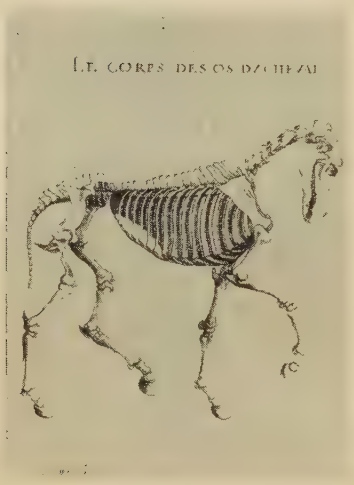

Fig. 5.

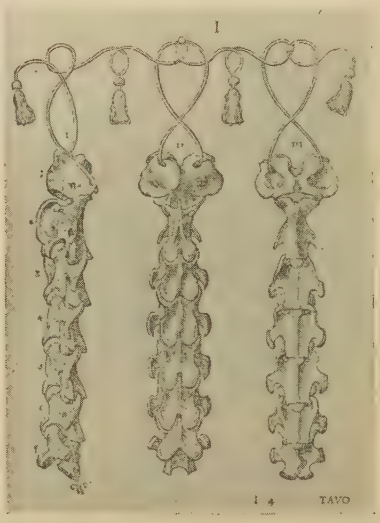

Fig. 6.

Leonardo and that these revealed on closer examination some errors in anatomical detail, due, according to Schmutzer, to Leonardo drawing from memory. A decision between these conflicting views has been much helped by a careful study on " Leonardo da Vinci, the Anatomist " by J. Playfair McMurrich (1930), in which the author has been able to study the question from the standpoint of a teacher of anatomy with a thorough knowledge of the relevant literature. In reading this book one is struck by finding that Leonardo had little or no classical learning and nevertheless he was influenced by several erroneous opinions in the anatomical treatise of Mondino de'Luzzi (1270-?1324), the only one he apparently knew. This smattering of knowledge brought him often in conflict with features he observed in his dissections and since we are dealing with a pioneer, it is not to be marvelled

that mistaken interpretations are frequently noticeable by the modern anatomist. Thus Leonardo represented a large blood vessel connecting liver and spleen. Another matter to be considered is that all Leonardo's anatomical work is recorded in his notebooks, which contain hastily jotted down observations extending over many years and which often refer to opinions which were eventually modified and corrected. Not only this but a peculiarity of Leonardo should be mentioned; that he allowed his imagination full play and would represent anatomical features, not so much as they were in reality but as they would be if their mechanical purpose were what he understood them to be. Thus he drew muscles as links or stays supporting schematised bones, and so forth. Moreover that he transposed organs, for example by drawing the larynx of a dog in the neck of a man, for purposes we can only surmise. These and many other points show that though he did intend preparing a treatise of anatomy and physiology, it was not medical anatomy he had in view, but rather he was investigating the subject from the standpoint of an artist and also a scientist. He thereupon reproduced human viscera, not because they might be the seat of disease, but because they supported the contour of the body and because they possessed functions about which he was curious. Moreover his artistic eye caused him to see and delineate his anatomical subjects as classical examples of athletic manhood and statuesque womanhood, which they can scarcely have been in reality.

McMurrich proves clearly that as an anatomist Leonardo da Vinci may be rightly considered as a scientific pioneer of the very first rank and that his influence on anatomical progress may not have been as limited as often supposed; that, for example, Albrecht Dürer (1471-1528) undoubtedly copied some of his drawings and that his influence on other contemporary artists must have been great, to judge from the oft quoted remarks of Giorgio Vasari (1512-1574). It is unfortunate that much of this is conjecture, however well documented, but the value and thoroughness of Leonardo as an anatomist had to be made clear for the purpose of explaining the connection with Ruini's drawings somewhat more convincingly than has been done by Jackschat and others.

Since Ruini does not mention the artist who obviously must have helped in the delineations, one may consider the suggestion of Schmutzer (1913); that a member of the "Accademia degli Incamminati" (freely translated: the "Academy of the advanced") and some anatomist from Bologna University helped Ruini in preparing the material and in making the drawings. Obviously these are suppositions and many other similar can be found in literature, but it is now possible to bring fresh information to bear on the problem.

On the basis of McMurrich's work there is now conclusive evidence that Leonardo became a skilled dissector and that in

connection with human anatomy he completed his study of the muscles by that of the inner organs and bones. Anyone who has seen some of the numerous sketches made by this truly remarkable Super-man, who with persistent assiduity in dozens and dozens of delineations reproduced drapery, flowing water and waves, facial expressions and many other fleeting appearances, will expect to find the movements and muscles of the horse together with the so-called "canons of proportion" represented in numerous designs, accompanied by notes in his neat mirror handwriting and colloquial Italian. These have been traced, but though the proportions of the horse are worked out with the utmost care and attention, the muscles traced and numbered, no designs of the inner organs have been found or recognised. A remarkable defect, and one cannot help thinking that they must have been made and were then appropriated by others.

A second point is that though Ruini states that he studied anatomy for the purpose of learning how to understand the treatment of the diseases of the horse, nevertheless, the drawings of the muscles of the horse are specifically artist's drawings, which consider the problems of foreshortening and delineation of movement, which are of no interest whatsoever to the anatomist.

Similar remarks apply to the osteology; for example, the reproduction of the vertebræ exhibits, it is true, absolute anatomical accuracy, but also an ornate knotting of ropes, together with a remarkable individuality of the tassels; this is absolutely in the style of Leonardo, who delighted in drawing for drawing's sake (see Fig. 6).

Then attention should be given to the necks of the horses on Plate V; these are abnormally thick, a feature which may be considered a distinctive mannerism of Leonardo, who intended the thick neck to balance the bulk of the body of the rider, for which the horse drawings were intended. (See Figs. 7 and 8.).

As an example of the fineness of details in some of the illustrations Plate I illustrating the nervous system of the horse may be reproduced; it may be doubted whether at the time any anatomist existed who was capable of giving such an accurate representation of the nervous system of any animal. (See Fig. 9.).

These objective observations seem to indicate that the illustrations of Ruini's anatomy of the horse may well have been based on sketches and drawings made by Leonardo and then scattered in his travels. A period of a hundred years would pass between the time of their execution and their publication and in the meanwhile the outstanding work of Vesalius, " De fabrica," would have appeared in 1543; this was definitely an anatomical work on medical principles and which as McMurrich points out, contains a mistaken delineation of the pelvis, which is correct in Leonardo's sketches. That Ruini was able to absorb medical knowledge can be seen in many pages of the text, which one may

concede was his, unless definite evidence to the contrary is forthcoming. Therefore it is difficult to understand why he should have adopted the artist's point of view in dealing with the

Fig. 7.

Fig. 8.

Fig. 9.

anatomy of the horse, unless he found the drawings ready-made in that style.

Before leaving the subject it may be mentioned that Leonardo

was intensely interested in the problem of what was eventually ascertained to be the circulation of the blood, though his anatomical dissections of the heart did not satisfy him that the Galenical doctrine of the blood movement was correct. He attempted to calculate the number of heart beats per hour and the volume of blood, both reckonings being inaccurate, one surmises through lack of suitable instruments. His philosophical outlook led him astray by suggesting the analogy of the movement of the waters from mountains along rivers to the sea; as an example of his trend of thought one may quote: "The origin of the sea is the reverse of that of the blood, for the sea receives into itself all the rivers which are totally produced from the aqueous vapours that have ascended into the air, while the sea of blood is the source of all veins" (from McMurrich An.A4). Some authors, like Holl (1911), Castiglioni (1934), hold that Leonardo came near discovering the circulation of the blood; admittedly, but not as near as Ruini. Therefore the source of Ruini's knowledge in this respect must be ascertained elsewhere.

One may therefore conclude that whilst the text of the book may have been compiled and written by Ruini, the drawings must have been founded on the work of a skilful anatomist working with a supreme craftsman. From the evidence of the sketches that remain and the style of the plates it can be suggested that Leonardo da Vinci's drawings served as a basis for a great part of those reproduced.

Real conclusive evidence probably will never be forthcoming, but if one sees the notable contribution made by McMurrich to the controversial question whether Vesalius borrowed anything from Leonardo or not, it may be possible in time to find the link between Senator Carlo Ruini and Leonardo da Vinci and decide how much the lawyer owed to the artist and scientist.

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SYNGAMUS TRACHEA. THE LONGEVITY OF THE INFECTIVE LARVÆ IN THE EARTHWORM. SLUGS AND SNAILS AS INTERMEDIATE HOSTS.

By E. L. Taylor.

Veterinary Laboratory, Ministry of Agriculture and Fisheries.

Early investigators were of the opinion that the earthworm acted as an intermediate host for the gapeworm of chickens, *Syngamus trachea,* but it is only recently that this point has been established with certainty. In 1928 Cram made a mention of having found the larvæ of this parasite in the musculature of an earthworm, the significance of which was discussed by Taylor (1930), but the details were not worked out until 1934 when Clapham published an account of her observations.

An investigation into the longevity of the infective larvæ in the earthworm was commenced at this laboratory in 1931 and has already given some interesting results which are recorded in the present note.

The method which has been followed has been to infect a small plot of ground very heavily with infective *Syngamus* eggs and larvæ and afterwards, at varying intervals, to dig up earthworms and feed them to experimental chicks. For this purpose a large dose of the infective eggs and larvæ of *Syngamus trachea* was given to three adult turkeys. This resulted in an infestation which was so heavy that, in spite of the large size of the trachea of these birds, respiration was seriously hampered and a little extra exertion soon resulted in distressed breathing; inspection through the glottis showing the inner wall of the trachea to be literally covered with the worms.

These heavily infested turkeys were confined to a small pen, only five yards long and four yards wide, between May 8th, 1931, and June 5th, 1931, and during that time passed large numbers of *Syngamus* eggs on to the ground. On two or three occasions during these four weeks the surface of the ground was turned over with a spade, and the first experimental feeding of the earthworms to chicks, for the purpose of testing the longevity of the infection, was carried out on April 18th, 1932. The following table gives the particulars of the experimental feedings and the results which were obtained.

Table I.

Showing Particulars of the Feeds of Infective Earthworms, and the Results Obtained.

Date when earthworms were fed to chicks.	*Age of infection in days.*	*Number of earthworms used.**	*Number of chicks receiving earthworms.*	*Pairs of gapeworms which developed in the individual chicks.*
18.4.32	317	5†	2	1, 0.
26.4.32	325	One to each chick‡	12	24, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0.
27.5.32	356	Two to each chick‡	6	2, 0, 0, 0, 0, 0.
1.6.32	361	One to each chick‡	12	3, 1, 1, 1, 4, 0, 0, 0, 0, 0, 0, 0.
13.8.32	430	200	21	1, 2, 8, 6, 1, 7, 8, 3, 1, 1, 1, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0.
21.1.33	596	20	5	1, 1, 1, 0, 0.
6.2.33	612	100	5	1, 2, 0, 0, 0.
14.6.33	689	140	3	15, 13, 0.
21.12.33	924	30	3	0, 0, 0.
9.2.34	975	180	3	27, 2, 0.
14.6.34	1,102	120	2	12, 1.
3.12.34	1,274	80	2	2, 2.

* Earthworms taken from the experimental pen have been identified as *Allolobophora longa* Ude, and *Allolobophora caliginosa* (Savigny).

† This represents the remains of a peptic digest of five earthworms from which the cyst shown in the figure had been taken.

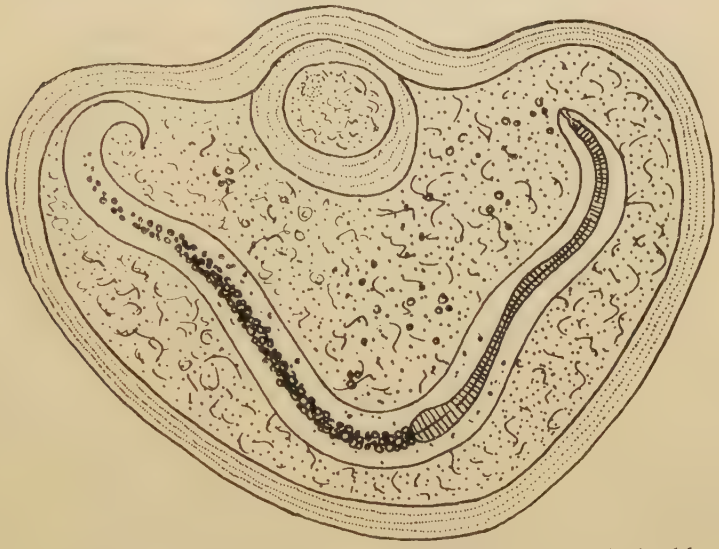
‡ These earthworms were kept in daily changes of wet blotting paper for eight days before they were fed to chicks. The remainder were taken direct from the pen and given to the chicks, as it was evident that the infection was not merely conveyed in soil contained in the worms.

Cyst Wall enclosing the Larvæ.

A search was made for the larvæ by digesting five earthworms taken from the infected pen 317 days after the deposition of infective material had ceased, and actively moving *Syngamus* larvæ were found in this material. One apparently viable larva was aslo found enclosed in a thick cyst wall, its morphology, as far as could be seen, corresponding in every way with that of the gapeworm larva. Other cysts were also found in this digest, containing what appeared to be *Syngamus* larvæ in various stages of degeneration, ranging from those in which nematode larvæ were easily recognisable down to mere granules enclosed within the clear substance of the thick cyst wall. Unfortunately, the tube containing these specimens was upset by an assistant and they were lost before a proper examination had been made or photographic record taken, and a second heavily infected earthworm containing these interesting stages has not been found among the several which have been examined. Clapham (1934) observed only thin-walled cysts in earthworms which had been infected for only a short time, and it is probable that the thickening of the wall is a gradual process.

Controls.

In order to ascertain whether the soil contained many infective larvæ and to rule out the possibility of infection being conveyed in a passive way by the earthworm, two chicks each received on each of five successive days one gramme of mixed worm castings from the infection pen, and each of two others received one gramme of soil, scraped from the surface of the pen on each of five successive days. None of the chicks, however, developed gapeworm infection, and keeping the test earthworms in wet blotting paper, as had previously been done, to rid them of soil before feeding them to chicks, was afterwards discontinued.



Larva of *Syngamus trachea* enclosed in a thick-walled cyst, obtained by digestion from an earthworm.

As *Syngamus* is of frequent occurrence in wild birds, the possibility of fresh infection being conveyed by them to the ground in the experimental pen had to be considered. Earthworms were therefore collected between February 9th and March 1st, 1934, from (*a*) the centre of the field adjoining the experimental pen, (*b*) under a large oak tree in the hedge surrounding the field, where the droppings of wild birds might be expected to reach the ground in larger amounts, and (*c*) from the experimental pen. Ten earthworms from each of these localities were collected on each of 18 consecutive days and fed to three groups of three experimental chicks. Each group of chicks thus received 180 earthworms, but when they were examined on March 15th only those which had received worms from the experimental pen proved to be infected; one of these contained 27 pairs, another contained two pairs and the third was uninfected.

This control was repeated in May, when 75 earthworms from under the oak tree and 150 from the centre of the field were fed to two batches of three chicks, but without resulting in any infection.

As a further control, on March 15th, 1935, two chicks received 100 earthworms collected from the soil six feet away from the experimental pen on the one side where some adult fowls had been kept in coops; and two other chicks received 100 earthworms from the soil six feet away from the other side of the experimental pen. When these chicks were killed, three weeks after the feeding of the last earthworms, they gave a negative result.

Discussion.

These observations seem to justify the conclusion that the infection in the experimental pen must have originated from the turkeys, and the larvæ are thus shown to have retained their vitality in the earthworms for three years and six months.

The larvæ of *Syngamus* are extraordinarily resistant in some ways, and can even develop to the infective stage when the eggs are incubated in the putrefying parent worms. Rise (1929) obtained infective larvæ for experimental purposes by allowing the worms to disintegrate under moist conditions at room temperature, and observations at this laboratory show that infective larvæ can actually be obtained in this way.

An interesting observation was also made on the resistance of the infective larvæ within the earthworm. The soil in a flowerpot in which some infected brandlings, *Eisenia fœtida*, had been kept was found to have become dry, and the two contained brandlings to be dead, and in a very dry shrivelled and brittle condition. These two worms were fed to two young chicks, as a result of which each chick developed one pair of gapeworms.

The longevity of the larvæ on the ground appears, however, to be rather below than above the average for what is known of most strongyloid worms; soil taken from the infected pen 334 days after the infected turkeys had been taken out failed to produce infection, and it has been noticed that even when stored in moist sand in the cold room at about 40° F. the larvæ die within eight months. The earthworm appears, therefore, to act, not only as a collector of infective larvæ as has previously been discussed (Taylor, 1930), but also as a very efficient storing agent.

From a taxonomic point of view the discovery that this nematode is capable of utilising an intermediate host is of some interest, as it suggests biological affinities with the Metastrongylidæ; this being strengthened by the situation of the worms in the respiratory apparatus, and by the structure of the bursa of the male.

Slugs and Snails as Intermediate Hosts.

There are several reasons for thinking that the gapeworm might be able to utilise intermediate hosts other than the earthworm as storage agents and means of conveyance to the final host. The ability of this parasitic nematode to reach the infective stage in the free-living state suggests that its intermediate-host requirements may not be very exacting; and reports of its occurrence in the swift (Cobbold, 1879) suggest that the larvæ might even be carried by flies.

The two spirurid worms *Physocephalus sexalatus*, found in the stomach of the pig, and *Spirocerca sanguinolenta* from the walls of the œsophagus and stomach of the dog are, perhaps, the most striking examples of the faculty of some nematodes to use a variety of hosts. The infective larval stages of these worms occur in various amphibia, reptilia, birds and mammals, as well as in the several species of beetles which normally serve as intermediate hosts.

If it is true that *Syngamus* has close affinities with the Metastrongylidæ it may not be too far removed systematically from these spirurids to look for some similarities in the life histories.

The series of feeding experiments here reported have therefore, been carried out to determine whether some common field invertebrates other than earthworms are capable of serving as agents for the storage and for the transmission of *Syngamus* to chickens. In a preliminary experiment a variety of small creatures were placed in glass vessels in the laboratory along with cabbage leaves and the roots of grasses thoroughly contaminated with eggs and larvæ of *Syngamus trachea*. They were kept there for 14 days; then daily for twelve days following this infection period they were placed on clean moist blotting paper in clean glass vessels, the slugs and snails also being washed daily in water. This was done with the idea of removing any infective material which might have been clinging on to the outside of the creatures and to allow adequate time for the elimination of infective material from their intestines.

At the end of this twelve days cleansing period they were given to test chickens, as a result of which it was found that one of the three chickens, which received the ten slugs and one snail between them, contained 28 pairs of gapeworms. The two other chickens gave negative results, as also did those which received the two caterpillars, 32 millipedes, four leather jackets, twelve click beetles, six wireworms and four woodlice, which had been treated in the same way.

Experimental Infection of Snails.

The experiment was then repeated with the large garden snail *Helix aspersa* and the common grey slug *Agliomax agrestis*.

The general method employed in this and in all subsequent

feeding experiments with slugs and snails has been to place the infective eggs and larvæ of *Syngamus* in small drops of water on to the surface of lettuce and cabbage leaves, and to allow the water to evaporate; then to place the leaves in large covered glass dishes, on the floors of which a layer of damp blotting paper had been placed. In this moisture-saturated atmosphere the leaves retained their freshness for several days, and usually long enough for the slugs and snails to eat the greater part.

After feeding on several lots of this material the slugs and snails were washed and removed to a clean glass vessel containing clean blotting paper, daily washing of the snails and changing of the blotting paper being carried out for as many as twelve days (in the early experiments) before finally feeding the slugs and snails to experimental chickens.

On this occasion each of nine experimental chicks received one slug (*A. agrestis*), and on subsequent autopsy it was found that each of two of the nine chicks contained one pair of *Syngamus*, the other seven chicks being uninfected. The eight *Helix aspersa*, which had been exposed to infection, were roughly dissected into visceral parts and muscular parts and fed to chicks. Four of the eight chicks which received the muscular parts were subsequently found to contain one, three, four and six pairs of gapeworms respectively. Six of the eight which received the visceral parts were found to be infected and to carry two, three, six, eight, ten and 17 pairs of gapeworms respectively.

Other species of snails were next tried in the same way and positive results were obtained with *Cepea hortensis* and *Helicella heripensis*, but infection was not transferred by the only two specimens of *Trichia hispida* which were tried.

The most successful infections were with *Helicella heripensis*; the feeding of one of these snails led to the development of 21 pairs of gapeworms in a chick; one *Agriolimax agrestis* fed to a chick resulted in the development of 20 pairs of gapeworms, and one *Helix aspersa* to the development of 17 pairs of worms. Other feeding experiments resulted in lighter infections.

Longevity of Infection in the Snail.

There has been no opportunity to learn much about the period which these intermediate hosts are capable of carrying infection, but an attempt is being made to get some information on that point and up to the present the longest observed period is seven months; one *H. aspersa* which had been infected six months previously resulted in the development of six pairs of gapeworms, and one *Cepea hortensis* infected seven months previously led to the development of three pairs of gapeworms in an experimental chick.

Situation of the Larvæ in the Snail.

An endeavour was next made to ascertain in what part of these intermediate hosts the larvæ take up their position. The rough dissections of *H. aspersa* mentioned above suggested that most of the larvæ were to be found in the visceral parts of that species; but subsequent examination of crush preparations of infected *A. agrestis* showed that by far the greater proportion were to be found in parts which did not come away with the viscera in a rough dissection, and particularly in the head. In the examination of four infected slugs by this method only four larvæ were found in the visceral parts, whereas 58 were found in the other more solid parts, including the foot, body-wall and head.

Serial sections were next prepared of one slug which had been subjected to particularly heavy infection and a rather unusual distribution of the larvæ was seen. Three were found in the tissues of the body-wall, one in the foot, one in the body cavity, one in the hermaphrodite gland, one in the liver, one in the salivary gland and 15 in the nerve ganglia of the head.

The larvæ were not enclosed in any kind of a cyst wall, but appeared to be lying free in the tissues, the positions in which they were found suggesting that they might have been actively wandering. No sign was observed of any reaction in the tissues of the intermediate host.

Serial sections were subsequently made of two *H. aspersa* which had been infected six months previously, as it was thought that by this time the larvæ might have become encysted. Only one larva was found, however, and that one appeared to be lying free in a salivary gland.

Flies.

The reported finding of *Syngamus trachea* in the swift, which presumably never feeds on the ground, suggested that it would be worth while looking into the possibility of flies acting as intermediate hosts. Seven lots of *Musca domestica* and *Stomoxys calcitrans* were therefore bred in the laboratory, in horse fæces containing a large number of the infective eggs and larvæ of *S. trachea*. Four lots of bluebottle flies (*Lucilia*) were also reared in contact with many infective eggs and larvæ of *Syngamus*, in hard-boiled eggs in the laboratory. Washed larvæ, pupæ and adult flies from these cultures were afterwards fed to young chicks but in no instance did infection result.

Other Possible Intermediate Hosts.

Further attempts were made to transmit infection by means of several other common field invertebrates, caterpillars, leather jackets, wireworms, millipedes, woodlice and one or two coprophagus beetles, but without success.

Summary and Conclusions.

Observations are reported which show that the larvæ of *Syngamus trachea* retain their infectivity (for the chick) in earthworms for upwards of three-and-a-half years.

The genus *Syngamus* probably has close affinities with the Metastrongylidæ.

Feeding experiments with land molluscs show that *Agriolimax agrestis, Helix aspersa, Cèpea hortensis* and *Helicella heripensis* are capable of acting as intermediate hosts in a similar way to the earthworm.

The larvæ were found to be situated in several different tissues but in *A. agrestis* there appears to be a preference for the nerve ganglia, out of a total of 23 larvæ found in one of these slugs 15 were observed to be situated in the nerve ganglia of the head.

Feeding experiments with three species of flies, and with a variety of other possible intermediate hosts did not result in infection.

Measures directed at the control of earthworms, slugs and snails are indicated where it is desired to eradicate *S. trachea* from restricted premises.

Acknowledgment.

I wish gratefully to acknowledge the kindness of Mr. C. C. A. Monroe in identifying the earthworms, and the help of Mr. G. C. Robson, also of the South Kensington Museum, who very kindly determined the species of slugs and snails and helped with the identification of some of the tissues in which the larvae were found.

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Erratum.

In the article on Johne's disease in the previous number of the Journal, page 136, the second + sign in the Table should have been —.

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,, ,,	Charcoal	5/-	4/6 ,,
,, ,,	Chinosol, ½ drm.	8/6	8/- ,,
,, ,,	Creoform	8/6	8/- ,,
,, ,,	Creosalgen Surgical, 3 per cent. ...	5/-	4/6 ,,
,, ,,	Formaldehyde, 2 per cent.	6/-	5/6 ,,
,, ,,	Hyd. Perchlor. (1 in 2000) ...	5/6	5/- ,,
,, ,,	Iodermiol, 10 per cent.	7/6	7/- ,,
,, ,,	Iodoform, 1 drm.	7/-	6/6 ,,
,, ,,	Lysalform	6/-	5/6 ,,
,, ,,	Lysoline	5/6	5/- ,,
,, ,,	Salol, 2 drms.	7/-	6/6 ,,
,, ,,	Salol, 2 drms. Iodoform, 1 drm.	8/9	8/3 ,,

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